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(54) Title: HUMAN CTLA-8 AND USES OF CTLA-8-RELATED PROTEINS

CambridgePark Drive, Cambridge, MA 02140 (US).

(57) Abstract

Polynucleotides encoding human CTLA-8 and related proteins are disclosed. Human CTLA-8 proteins and methods for their production are also disclosed. Methods of treatment using human CTLA-8 proteins, rat CTLA-8 proteins and herpesvirus herpes CTLA-8 proteins are also provided.

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HUMAN CTLA-8 AND USES OF CTLA-8-RELATED PROTEINS

This application is a continuation-in-part of application Ser. No. 08/504,032, filed July 19, 1995, and a continuation-in-part of application Ser. No. 08/514,014, filed August 11, 1995.

Field of the Invention

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The present invention relates to human CTLA-8 proteins, nucleic acids encoding such proteins, methods of treatment using such proteins. The invention also relates to the use of rat CTLA-8 proteins and herpesvirus *Saimiri* ORF13 proteins in methods of treatment.

Background of the Invention

Cytokines are secreted proteins which act on specific hematopoietic target cells to cause a differentiation event or on other target cells to induce a particular physiological response, such as secretion of proteins characteristic of inflammation. Cytokines, also variously known as lymphokines, hematopoietins, interleukins, colony stimulating factors, and the like, can be important therapeutic agents, especially for diseases or conditions in which a specific cell population is depleted. For example, erythropoietin, G-CSF, and GM-CSF, have all become important for treatment of anemia and leukopenia, respectively. Other cytokines such as interleukin-3, interleukin-6, interleukin-11 and interleukin-12 show promise in treatment of conditions such as thrombocytopenia and modulation of immune response.

For these reasons a significant research effort has been expended in searching for novel cytokines and cloning the DNAs which encode them. In the past, novel cytokines were identified by assaying a particular cell such as a bone marrow cell, for a measurable response, such as proliferation. The search for novel cytokines has thus been limited by the assays available, and if a novel cytokine has an activity which is unmeasurable by a known assay, the cytokine remains undetectable. In a newer approach, cDNAs encoding cytokines have been detected using the polymerase chain

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reaction (PCR) and oligonucleotide primers having homology to shared motifs of known cytokines or their receptors. The PCR approach is also limited by the necessity for knowledge of previously cloned cytokines in the same protein family. Cytokines have also been cloned using subtractive hybridization to construct and screen cDNA libraries, or they can potentially be cloned using PCR followed by gel electrophoresis to detect differentially expressed genes. The subtractive hybridization methods are based on the assumption that cytokine mRNAs are those that are differentially expressed, and these methods do not require any prior knowledge of the sequence of interest. However, many cytokines may be encoded by mRNAs which are not differentially expressed, and thus are undetectable using these methods.

It would be desirable to develop new methods for identifying novel cytokines and other secreted factors and to isolate polynucleotides encoding them.

Summary of the Invention

In developing the present invention, methods were employed which selectively identify polynucleotides which encode secreted proteins. One such polynucleotide was isolated which encodes "human CTLA-8." In accordance with the present invention, polynucleotides encoding human CTLA-8 and active fragments thereof are disclosed. "CTLA-8" is used throughout the present specification to refer to both proteins and polynucleotides encoding those proteins and to refer to proteins and polynucleotides from all mammalian species.

In certain embodiments, the present invention provides an isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:

- (a) the nucleotide sequence of SEQ ID NO:1 from nucleotide 146 to nucleotide 544;
- (b) a nucleotide sequence capable of hybridizing to a nucleic acid sequence specified in (a);
- (c) a nucleotide sequence varying from the sequence of the nucleotide sequence specified in (a) as a result of degeneracy of the genetic code; and
 - (d) an allelic variant of the nucleotide sequence specified in (a).

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Preferably, the polynucleotide of the invention encodes a protein having CTLA-8 activity. In other embodiments the polynucleotide is operably linked to an expression control sequence. In other preferred embodiments, the polynucleotide is contained in a vector suitable for *in vivo* expression in a mammalian subject. Polynucleotides comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 55 to nucleotide 544, the nucleotide sequence of SEQ ID NO:1 from nucleotide 139 to nucleotide 544 or the nucleotide sequence of SEQ ID NO:1 from nucleotide 86 to nucleotide 544 are particularly preferred.

Host cells transformed with the polynucleotides of the invention are also provided, including mammalian cells.

Processes are also provided for producing a human CTLA-8 protein, said processes comprising:

- (a) growing a culture of the host cell of the invention in a suitable culture medium; and
- (b) purifying the human CTLA-8 protein from the culture.

 Isolated human CTLA-8 protein is also provided which comprising an amino acid sequence selected from the group consisting of:
 - (a) the amino acid sequence of SEQ ID NO:2;
 - (b) the amino acid sequence of SEQ ID NO:2 from amino acids 11 to 163;
 - (c) the amino acid sequence of SEQ ID NO:2 from amino acids 29 to 163;
- (d) the amino acid sequence of SEQ ID NO:2 from amino acids 31 to 163; and
 - (e) fragments of (a), (b), (c) or (d) having CTLA-8 activity.
- 25 Proteins comprising the amino acid sequence of SEQ ID NO:2 and comprising the sequence from amino acids 29 to 163, from amino acid 31 to 163, or from amino acids 11 to 163 of SEQ ID NO:2 are particularly preferred. Preferably, the protein has CTLA-8 activity. Pharmaceuticals composition comprising a human CTLA-8 protein of the invention and a pharmaceutically acceptable carrier are also provided.
 - Compositions are also disclosed which comprise an antibody which specifically reacts with a human CTLA-8 protein of the invention.

Methods of treating a mammalian subject are also provided which comprise administering a therapeutically effective amount of a pharmaceutical composition comprising a human CTLA-8 protein.

Rat CTLA-8 and active (i.e., having CTLA-8 activity) fragments thereof may also be used in such methods of treatment. Preferably the rat protein is administered as a composition comprising a pharmaceutically acceptable carrier and a protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:4;

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- (b) the amino acid sequence of SEQ ID NO:4 from amino acids 18 to 150; and
- (c) fragments of (a) or (b) having CTLA-8 activity.

Herpesvirus Saimiri ORF13, referred to herein as "herpes CTLA-8", and active (i.e., having CTLA-8 activity) fragments thereof and active fragments thereof may also be used in such methods of treatment. Preferably the herpes CTLA-8 protein is administered as a composition comprising a pharmaceutically acceptable carrier and a protein comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:6;
- (b) the amino acid sequence of SEQ ID NO:6 from amino acids 19 to 151;and
- (c) fragments of (a) or (b) having CTLA-8 activity.

The invention also provides a method of treating a mammalian subject comprising administering a therapeutically effective amount of a composition comprising a pharmaceutically acceptable carrier and IL-17 or an active fragment thereof.

In methods of treatment provided by the present invention, preferably the subject is treated to produce an effect selected from the group consisting of inhibition of angiogenesis, inhibition of growth or proliferation of vascular endothelial cells, inhibition of tumor growth, inhibition of angiogenesis-dependent tissue growth, proliferation of myeloid cells or progenitors, proliferation of erythroid cells or progenitors, proliferation of lymphoid cells or progenitors, induction of IFNγ production, induction of IL-3 production and induction of GM-CSF production.

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Brief Description of the Figures

Fig. 1 is a comparison of homologous regions of the amino acid sequences of human CTLA-8 (indicated as "B18_F1"), rat CTLA-8 (indicated as "Musctla8") and herpes CTLA-8 (indicated as "Hsvie_2").

Fig. 2 depicts autoradiographs demonstrating expression of human CTLA-8 in COS cells.

Fig. 3 presents data relating to the ability of human CTLA-8 to inhibit angiogenesis.

Figs. 4 and 5 present data relating to the ability of human CTLA-8 to produce or induce hematopoietic activity.

Figs. 6 and 7 present data demonstrating the ability of human CTLA-8 to induce production of IL-6 and IL-8.

Detailed Description of Preferred Embodiments

The inventors of the present application have identified and provided a polynucleotide encoding a human CTLA-8 protein. SEQ ID NO:1 provides the nucleotide sequence of a cDNA encoding the human CTLA-8 protein. SEQ ID NO:2 provides the amino acid sequence of the human CTLA-8 protein. Alternatively, the initiating methionine may be at amino acid 11 of SEQ ID NO:2. On the basis of amino terminal sequencing, the mature protein sequence is believed to begin at amino acid 31 of SEQ ID NO:2 (encoded by the sequence beginning with nucleotide 146 of SEQ ID NO:1).

The region from amino acid 29 to amino acid 163 of human CTLA-8 (SEQ ID NO:2) shows marked homology to portions of rat CTLA-8 (amino acids 18 to 150 of SEQ ID NO:4) and herpesvirus *Saimiri* ORF13 ("herpes CTLA-8") (amino acids 19 to 151 of SEQ ID NO:5). A cDNA sequence encoding rat CTLA-8 is listed at SEQ ID NO:3 and its corresponding amino acid sequence is reported at SEQ ID NO:4. A cDNA sequence encoding herpes CTLA-8 is listed at SEQ ID NO:5 and its corresponding amino acid sequence is reported at SEQ ID NO:6. Homology between rat CTLA-8 and herpes CTLA-8 was reported by Rouvier et al., J. Immunol. 1993, 150, 5445-5456.

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Applicants had previously incorrectly identified the rat sequences of SEQ ID NO:3 and SEQ ID NO:4 as applying to murine CTLA-8. Applicants' human CTLA-8 (B18) does also show homolgy to the true murine CTLA-8 sequence.

Golstein et al. (WO95/18826; Fossiez et al., Microbial Evasion and Subversion of Immunity 544:3222 (Abstract)) have also reported a species they initially identified as "human CTLA-8." However, examination of the sequence of the Golstein et al. species and the human CTLA-8 (B18) sequence of the present invention readily reveals that they are two different proteins, although they are homologous with each other and with the rat CTLA-8 and herpes CTLA-8 identified herein. The Golstein et al. species has now been renamed as interleukin-17 (IL-17). Because of the homology between applicants' human CTLA-8 (B18) and IL-17, these proteins are expected to share some activities.

It has also been preliminarily determined that human CTLA-8 (B18) forms homodimers when expressed. As a result, human CTLA-8 proteins may possess activity in either monomeric or dimeric forms. Human CTLA-8 proteins can also be produced as heterodimers with rat and herpes CTLA-8 proteins and with human IL-17. These heterodimers are also expected to have activities of the proteins of which they are comprised.

Forms of human CTLA-8 protein of less than full length are encompassed within the present invention and may be produced by expressing a corresponding fragment of the polynucleotide encoding the human CTLA-8 protein (SEQ ID NO:1). These corresponding polynucleotide fragments are also part of the present invention. Modified polynucleotides as described above may be made by standard molecular biology techniques, including site-directed mutagenesis methods which are known in the art or by the polymerase chain reaction using appropriate oligonucleotide primers.

For the purposes of the present invention, a protein has "CTLA-8 activity" if it either (1) displays biological activity in a factor-dependent cell proliferation assay (preferably an assay in which full-length the corresponding species full-length CTLA-8 is active) (including without limitation those assays described below), or (2) induces expression or secretion of γ -IFN, or (3) displays chemoattractant of chemotactic activity in a chemoattraction or chemotaxis assay (preferably as assay in which full-

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length the corresponding species full-length CTLA-8 is active) or (4) induces expression of secretion of IL-3 or GM-CSF.

Human CTLA-8 protein or fragments thereof having CTLA-8 activity may be fused to carrier molecules such as immunoglobulins. For example, human CTLA-8 protein may be fused through "linker" sequences to the Fc portion of an immunoglobulin.

The invention also encompasses allelic variations of the nucleotide sequence as set forth in SEQ ID NO:1, that is, naturally-occurring alternative forms of the isolated polynucleotide of SEQ ID NO:1 which also encode human CTLA-8 or CTLA-8 proteins having CTLA-8 activity. Also included in the invention are isolated polynucleotides which hybridize to the nucleotide sequence set forth in SEQ ID NO:1 under highly stringent (0.2xSSC at 65°C), stringent (e.g. 4xSSC at 65°C or 50% formamide and 4xSSC at 42°C), or relaxed (4xSSC at 50°C or 30-40% formamideand 4xSSC at 42°C) conditions. Isolated polynucleotides which encode human CTLA-8 protein but which differ from the nucleotide sequence set forth in SEQ ID NO:1 by virtue of the degeneracy of the genetic code are also encompassed by the present invention. Variations in the nucleotide sequence as set forth in SEQ ID NO:1 which are caused by point mutations or by induced modifications which enhance CTLA-8 activity, half-life or production level are also included in the invention.

The isolated polynucleotides of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman et al., Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the CTLA-8 protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, Methods in Enzymology 185, 537-566 (1990). As defined herein "operably linked" means enzymatically or chemically ligated to form a covalent bond between the isolated polynucleotide of the invention and the expression control sequence, in such a way that the CTLA-8 protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

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A number of types of cells may act as suitable host cells for expression of the human CTLA-8 protein. Any cell type capable of expressing functional human CTLA-8 protein may be used. Suitable mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK, Rat2, BaF3, 32D, FDCP-1, PC12 or C2C12 cells.

The human CTLA-8 protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, e.g., Invitrogen, San Diego, California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. Soluble forms of the human CTLA-8 protein may also be produced in insect cells using appropriate isolated polynucleotides as described above.

Alternatively, the human CTLA-8 protein may be produced in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Suitable yeast strains include Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces strains, Candida, or any yeast strain capable of expressing heterologous proteins. Suitable bacterial strains include Escherichia coli, Bacillus subtilis, Salmonella typhimurium, or any bacterial strain capable of expressing heterologous proteins.

The human CTLA-8 protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a polynucleotide sequence encoding the human CTLA-8 protein.

The human CTLA-8 protein of the invention may be prepared by growing a culture of transformed host cells under culture conditions necessary to express the desired protein. The resulting expressed protein may then be purified from the culture medium or cell extracts. Soluble forms of the human CTLA-8 protein of the invention

can be purified from conditioned media. Membrane-bound forms of human CTLA-8 protein of the invention can be purified by preparing a total membrane fraction from the expressing cell and extracting the membranes with a non-ionic detergent such as Triton X-100.

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The human CTLA-8 protein can be purified using methods known to those skilled in the art. For example, the human CTLA-8 protein of the invention can be concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration medium. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred (e.g., S-Sepharose® columns). The purification of the human CTLA-8 protein from culture supernatant may also include one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; or by hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or by immunoaffinity chromatography. Finally, one or more reversephase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the human CTLA-8 protein. Some or all of the foregoing purification steps, in various combinations or with other known methods, can also be employed to provide a substantially purified isolated recombinant protein.

Preferably, the human CTLA-8 protein is purified so that it is substantially free of other mammalian proteins.

It is believed that human CTLA-8, active fragments and variants thereof, and CTLA-8 related proteins (such as, for example, rat CTLA-8 and herpes CTLA-8) (collectively "CTLA-8 proteins") possess or induce cytokine activities. Human

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CTLA-8 expression correlated with γ-IFN expression in induced primary cells and can induce the expression of IL-3 and/or GM-CSF, which expression can in turn produce effects associated with the induced cytokine. Therefore, human CTLA-8 and CTLA-8 related proteins may have an effect on proliferation or function of myeloid cells, erythroid cells, lymphoid cells and their progenitors. Human CTLA-8 proteins may also play a role in formation of platelets or their progenitors.

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnolli et al., J. Immunol. 145:1706-1712, 1990; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli, et al., J. Immunol. 149:3778-3783, 1992; Bowman et al., J. Immunol. 152: 1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A.M. and Shevach, E.M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human Interferon γ, Schreiber, R.D.

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1988.

In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L.S. and Lipsky, P.E. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988; Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human interleukin 6 - Nordan, R. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11 - Bennett, F., Giannotti, J., Clark, S.C. and Turner, K. J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9 - Ciarletta, A., Giannotti, J., Clark, S.C. and Turner, K.J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober
Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512,

A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various

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immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpes viruses, mycobacteria, leshmania, malaria and various fungal infections such as candida. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally would be indicated, i.e., in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, asthma and related respiratory conditions), may also be treatable using a protein of the present invention.

A protein of the present invention may also suppress chronic or acute inflammation, such as, for example, that associated with infection (such as septic shock or systemic inflammatory response syndrome (SIRS)), inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-1 (such as the effect demonstrated by IL-11).

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte

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Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowmanet al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: *In vitro* antibody production, Mond, J.J. and Brunswick, M. In *Current*

Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and

Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

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Dendritic cell-dependent assays (which will identify, among others, proteins expressed by denritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimenal Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine

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169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentarily to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility

in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either *in-vivo* or *ex-vivo* (i.e. in conjunction with bone marrow transplantation) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

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Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embyronic differentation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

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Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M.G. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I.K. and Briddell, R.A. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, NY. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R.E. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 1-21, Wiley-Liss, Inc.., New York, NY. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, NY. 1994; Long term culture initating cell assay, Sutherland, H.J. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, NY. 1994.

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CTLA-8 proteins are useful in the treatment of various immune deficiencies and disorders (including SCID), e.g., in regulating (up or down) growth, proliferation and/or activity of T and/or B lymphocytes, as well as the cytolytic activity of NK cells. These immune deficiencies may be caused by viral (e.g., HIV) as well as bacterial infections, or may result from autoimmune disorders. More specifically, infectious diseases caused by viral, bacterial, fungal or other infection may be treatable using CTLA-8 proteins, including infections by HIV, hepatitis, influenza, CMV, herpes, mycobacterium, leishmaniasis, malaria and various fungal infections (such as candida). Of course, in this regard, the CTLA-8 proteins may also be useful where a boost to the immune system generally would be indicated, i.e., in the treatment of cancer or as an adjuvant to vaccines. Autoimmune disorders which may be treated using factors of the present invention include, for example, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes melitis and autoimmune inflammatory eye disease. The CTLA-8 proteins are also expected to be useful in the treatment of allergic reactions and conditions.

CTLA-8 proteins are also expected to have chemotactic activity. A protein or peptide has "chemotactic activity," as used herein, if it can stimulate, directly or indirectly, the directed orientation or movement of cells, including myeloid and lymphoid cells. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells (particularly T-cells). Whether a particular protein or peptide has chemotactic activity for cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

CTLA-8 proteins also inhibit growth and proliferation of vascular endothelial cells. As a result, human CTLA-8 proteins are effective in inhibiting angiogenesis (i.e., vascular formation). This activity will also be useful in the treatment of tumors and other conditions in which angiogenesis in involved. Inhibition of angiogenesis by human CTLA-8 proteins will also result in inhibition or prevention of the condition to which normal angiogenesis would contribute.

Isolated CTLA-8 proteins, purified from cells or recombinantly produced, may be used as a pharmaceutical composition when combined with a pharmaceutically

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acceptable carrier. Such a composition may contain, in addition to CTLA-8 protein and carrier, diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-15, G-CSF, γ-IFN, stem cell factor, and erythropoietin. The pharmaceutical composition may contain thrombolytic or anti-thrombotic factors such as plasminogen activator and Factor VIII. The pharmaceutical composition may further contain other antiinflammatory agents. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with CTLA-8 protein, or to minimize side effects caused by the CTLA-8 protein. Conversely, CTLA-8 protein may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

The pharmaceutical composition of the invention may be in the form of a liposome in which CTLA-8 protein is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers which in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323, all of which are incorporated herein by reference.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, e.g., amelioration of symptoms of,

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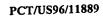
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healing of, or increase in rate of healing of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of CTLA-8 protein is administered to a mammal. CTLA-8 protein may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines, other hematopoietic factors or vaccine components (such as antigens or other adjuvants), CTLA-8 protein may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering CTLA-8 protein in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of CTLA-8 protein used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, or cutaneous, subcutaneous, or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of CTLA-8 protein is administered orally, CTLA-8 protein will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% CTLA-8 protein, and preferably from about 25 to 90% CTLA-8 protein. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol,



propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of CTLA-8 protein, and preferably from about 1 to 50% CTLA-8 protein.

When a therapeutically effective amount of CTLA-8 protein is administered by intravenous, cutaneous or subcutaneous injection, CTLA-8 protein will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to CTLA-8 protein an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additive known to those of skill in the art.

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The amount of CTLA-8 protein in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of CTLA-8 protein with which to treat each individual patient. Initially, the attending physician will administer low doses of CTLA-8 protein and observe the patient's response. Larger doses of CTLA-8 protein may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not generally increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.1 µg to about 100 mg of CTLA-8 protein per kg body weight, preferably about 0.1 µg to about 100 µg of CTLA-8 protein per kg body weight, more preferably about 0.1 µg to about 100 µg of CTLA-8 protein per kg body weight, most preferably preferably about 0.1 µg to about 100 µg of CTLA-8 protein per kg body weight, most preferably preferably about 0.1 µg to about 100 µg of CTLA-8 protein per kg body weight.

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The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the CTLA-8 protein will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

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CTLA-8 protein of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the CTLA-8 protein and which may inhibit CTLA-8 binding to its receptor. Such antibodies are also useful for performing diagnostics assays for CTLA-8 in accordance with known methods. Such antibodies may be obtained using the entire CTLA-8 protein as an immunogen, or by using fragments of human CTLA-8 protein. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Additional peptide immunogens may be generated by replacing tyrosine residues with sulfated tyrosine residues. Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, J.Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, et al., FEBS Lett. 211, 10 (1987).

Neutralizing or non-neutralizing antibodies (preferably monoclonal antibodies) binding to human CTLA-8 protein may also be useful therapeutics for certain tumors and also in the treatment of conditions described above. These neutralizing monoclonal antibodies are capable of blocking the ligand binding to the human CTLA-8 protein or mayh promote clearance of protein from the patient.

Because of their homology to human CTLA-8, rat CTLA-8 proteins, herpes CTLA-8 proteins and IL-17 proteins (the "human CTLA-8" of Golstein et al., supra) will also possess CTLA-8 activity as described above. As a result, rat and herpes CTLA-8 proteins and IL-17 proteins, as well as active fragments and variants thereof, can be used in preparation of pharmaceutical compositions and in methods of treatment as described for human CTLA-8. Rat and herpes CTLA-8 proteins, and active fragments and variants thereof, can be produced as described above using the polynucleotides (or fragments or variants thereof) described in SEQ ID NO:3 and SEQ ID NO:5, respectively. Rat and herpes CTLA-8 may also be produced as described in Rouvier et al., J. Immunol. 1993, 150, 5445-5456. CTLA-8 proteins of other

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species can also be used as described herein. cDNAs encoding rat CTLA-8 and herpes CTLA-8 were deposited with the American Type Culture Collection on July 6, 1995 and assigned accession numbers ATCC 69867 and ATCC 69866, respectively. IL-17 proteins may also be produced as described in Golstein et al., supra.

Because of its homology to IL-17, the human CTLA-8 (B18) proteins of the present invention may also share some activities with IL-17.

For the purposes of treatment or therapy, any of the proteins discussed or disclosed herein may be administered by *in vivo* expression of the protein in a mammalian subject. In such instances, a polynucleotide encoding the desired protein is administered to the subject in manner allowing expression in accordance with known methods, including without limitation the adenovirus methods disclosed herein.

Example 1

Isolation of Human CTLA-8 cDNA

A partial clone for human CTLA-8 was isolated from a cDNA library made from RNA isolated from stimulated human peripheral blood mononuclear cells. This partial was identified as "B18." B18 is sometimes used herein to refer to the human CTLA-8 of the present invention. Homology searches identified this partial clone as being related to the herpes and rat CTLA-8 genes. DNA sequence of this partial clone was used to isolate the full-length clone.

In order to isolate a full-length cDNA for B18, a directional, full-length cDNA library by standard means in the COS expression vector pMV2. The cDNA library was transformed into *E. coli* by electroporation. The bulk of the original transformed cDNA library was frozen in glycerol at -80°C. An aliquot was titered to measure the concentration of transformed *E. coli*. The *E. coli* were thawed, diluted to 76,000/0.1 ml in media containing ampicillin, and 0.1 ml was distributed into the wells of a microtiter dish in an 8 x 8 array. The microtiter dish was placed at 37°C overnight to grow the *E. coli*.

To prepare DNA for PCR, 20 µl aliquots of culture from each well were withdrawn and pooled separately for each row and column of eight wells, giving 16

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pools of 160 µl each. The *E. coli* were pelleted, resuspended in 160 µl of standard lysis buffer consisting of 10 mM TrisHCI pH8, 1 mM EDTA, 0.01% Triton X-100, and lysed by heating to 95°C for 10 minutes.

To identify which of the wells contained *E. coli* transformed with B18, PCR was performed first on the DNA preps corresponding to the eight columns. The PCR consisted of two sequential reactions with nested oligonucleotides using standard conditions. The oligonucleotides used for the PCR reaction were derived from the sequence of the partial B18 clone. They were:

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B185: CACAGGCATACACAGGAAGATACATTCA (SEQ ID NO:7)

B183: TCTTGCTGGATGGGAACGGAATTCA (SEQ ID NO:8)

B18N: ATACATTCACAGAAGAGCTTCCTGCACA (SEQ ID NO:9)

The PCR conditions were 2.5 mM $MgCl_2$ and 95°C x 2 min for one cycle, 95°C x 1 min plus 68°C x 1 min for 30 cycles, and 68°C x 10 for one cycle. Each reaction was 20 μ l. The first reaction contained oligonucleotides B185 and B183 and 1 μ l of the DNA preparations. The second reaction contained oligonucleotides B183 and B18N and 1 μ l of the first reaction.

DNA preps that potentially contained a full-length B18 cDNA clone were identified by agarose gel electrophoresis on an aliquot of the second PCR reaction. A DNA band of the correct mobility was assumed to be derived from a B18 cDNA. Next the same sequence of PCR reactions and gel analysis was done on the DNA preps corresponding to the eight rows. The intersection of a row and a column identified well A2 as potentially containing B18, narrowing it down to the 76,000 *E. coli* originally seeded into that well.

To further purify the individual *E. coli* containing the putative full-length B18 cDNA clone, the concentration of *E. coli* in well A2 was measured by titering and plating dilutions of the well. Then 7600 *E. coli* were seeded into the wells of a second microtiter plate in an 8 x 8 array. The *E. coli* were grown overnight; wells were pooled, and DNA was prepared as described above. To identify which of these wells contained *E. coli* transformed with B18, sequential PCR reactions were performed

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essentially as described above. Agarose gel electrophoresis identified well B2 as potentially containing a B18 cDNA.

The *E. coli* containing this cDNA was further purified by seeding wells of a microtiter plate with 253 *E. coli* per well and proceeding as for the purification of the *E. coli* in well A2. Well C3 was identified as containing a putative full-length B18 cDNA clone. The exact *E. coli* was identified by plating the contents of the well onto bacterial culture media and then screening the *E. coli* colonies following established protocols. The probe for these hybridizations was a PCR fragment generated by doing a PCR reaction on the B18 clone using as primers the oligonucleotides described above (SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9). When a single colony was identified, DNA was prepared and sequenced by standard methods. Comparison of this sequence to the sequence of the original partial clone confirmed identity and that the isolated cDNA was full-length.

The full-length clone was deposited with the American Type Culture Collection on July 6, 1995 and assigned accession number ATCC 69868.

Example 2

Expression of Human CTLA-8

The full-length B18 clone for human CTLA-8 was transfected into COS cells which were then labelled with ³⁵S-methionine. An aliquot of conditioned medium from the transfected cell culture was reduced, denatured and electrophoresed on polyacrylamide gels. Autoradiographs of those gels are reproduced in Fig. 2. The band indicated by the arrow demonstrates expression of human CTLA-8.

Example 3

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Inhibition of Angiogenesis by Human CTLA-8

The ability of human CTLA-8 to inhibit angiogenesis was examined in an angiostatic activity assay (endothelial cell proliferation assay). The assay was done in a 96 well plate. Primary human umbilical cells (HUVECs) were seeded to 2x10³ cells per well in EGM medium (Clonetics)/20% FCS and incubated at 37°C for 24 hr. The cells were then starved in M199 medium (GIBCO BRL) containing 10% charcoal

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treated serum (M199-CS) for 48 hr at 37°C. Conditioned media containing B18 (human CTLA-8) was obtained from transfected COS or stably expressing CHO cells and 1:10, 1:50, 1:250, and 1:1250 dilutions prepared in M199-CS medium containing 100 ng/ml FGF. The dilutions of B18 were added to the starved cells and incubated for 72 hr at 37°C. The cells were then radiolabeled by [³H]-thymidine for 6 hr. Radiolabeled cells were washed with PBS and trypsinized for liquid scintilation counting. Results were plotted using Kaleidograph software. The results are shown in Fig. 3. In the figure, "Med" is the mock control, "B18" and "B18-1" were conditioned medium from two independent transfections of COS with DNA encoding human CTLA-8 (B18). IFNγ was used as a positive control angiostatic (i.e., angiogenesis inhibition) activity. These data demonstrate that human CTLA-8 (B18) inhibits angiogenesis.

Example 4

Hematopoietic Activity of Human CTLA-8

The hematopoietic activity of human CTLA-8 (B18) expressed *in vivo* was examined by construction of a recombinant adenovirus vector.

The B18 cDNA in the expression plasmid Adori 2-12 B18 was driven by the cytomegalovirus(CMV) immediate early promoter and enhancer.

The Adori 2-12 vector was created by addition of an SV40 origin and enhancer to a known adenovirus vector (Barr et al., Gene Therapy 1:51 (1994); Davidson et al., Nature Genetics 3:219 (1993)). The HindIII/BamHI fragment encoding the SV40 origin and enhancer was isolated from the pMT2 mammalian expression vector, blunted with Klenow and cloned into the NatI site (blunted with Klenow) of the Ad5 expression vector.

The vector was derived by digesting pNOT-B18 cDNA with SalI, filling in the 5' overhang with Klenow to generate a blunt end and digesting with EcoRI to isolate the B18 cDNA. The blunted- EcoRI B18 fragment was inserted into the restriction sites EcoRV-EcoRI of the adenovirus vector Adori 2-12. The CMV-B18 expression cassette was located downstream of the SV40 origin and enhancer, and 0-1 map units of the left hand end of the adenovirus type 5(Ad5). The SV40 splice donor and

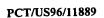
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acceptor were located between the CMV promoter and B18 cDNA. Following the insert was SV40 poly A site, 9-16 map units of Ad5 and the puc 19 origin.

A recombinant adenovirus was generated by homologous recombination in 293 cells. AscI linearized Adori 2-12 B18 and ClaI digested AdCMVlacZ were introduced into the 293 cells using lipofectamine. Recombinant adenovirus virus was isolated and amplified on 293 cells. The virus was released from infected 293 cells by three cycles of freeze-thawing. The virus was further purified by two cesium chloride centrifugation gradients and dialyzed against PBS 4°C. Following dialysis of the virus glycerol was added to a concentration of 10 % and the virus was stored at - 70 °C until use. The virus was characterized by expression of the transgene, plaque forming units on 293 cells, particles/ml and Southern analysis of the virus.

A single dose of 5 X 10 ¹⁰ particles of recombinant adenovirus encoding B18 was injected into the tail vein of male C57/bl6 mice, age 7-8 weeks. Control mice received an adenovirus encoding B-galactosidase. Four mice from each experimental group were killed on day 7 and 14. Blood was collected and automated hematologic analysis was performed using a Baker 9000. Differential counts were performed on blood smears. Tissue was harvested, fixed in formalin, and stained with hematoxylin and eosin for histopathology. In the first set of experiments, serum and tissues were analyzed 7 and 14 days post injection. A slight increase in peripheral platelet counts were observed. The animals that received B18 exhibited a slight increase in spleen size. Macroscopic analysis of the spleen showed an increase in splenic extramedullary hematopoiesis on day 7 compared to the control. These results showed a hematopoietic growth activity associated with B18.

In a second set of experiments 5 X 10 ¹⁰ particles of recombinant adenovirus encoding B18 were injected into the tail vein of male C57/bl6 mice, age 17-18 weeks. Control mice received an adenovirus encoding B-galactosidase. Blood samples were collected via retro-orbital sinus on days 2, 5, 7, 10, 14, and 21. The hematologic analyses were performed on the Baker 9000 automated cell counter with murine-specific settings. Analyses included WBC, RBC, HCT and PLT counts. Blood smears were prepared and stained with Wright-Geimsa for WBC differentials based on a 100 cellcount. Reticulocytes and reticulated platelets were quantitated using flow

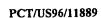
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cytometry. Four mice from each group were killed on days 7, 14, and 21. In addition to peripheral blood analysis, serum was collected via cardiac puncture for quantitation of systemic II-6 using a commercial kit (Endogen). Spleen and liver were collected for histopathology, spleen and bone marrow hematopoietic progenitors were quantitated, and bone marrow smears were prepared and stained with Wright-Geimsa for cell counts.

Administration of adenovirus encoding B18 resulted in a marked increase in peripheral blood neutrophils and WBC (Fig. 4). Maximum increases in neutrophils were observed at day 5 and day 7. The control mice showed little difference at day 5 and day 7. Peripheral blood neutrophils were similar in the control mice and mice that received B18 at day 21. In both the B18 and control groups an increase in white blood cells was also observed. The mice that received B18 had a greater increase in WBC between day 2 and day 7. By Day 21 a more pronounced increase was observed in the B-gal group. No other changes in cellular chemistries were observed (Table I).

Bone marrow cellularity was calculated from pooled femurs in each group (Table III). No significant differences were observed in either group. No significant changes were observed in bone marrow hematopoietic progenitors from day 7, 14, and 21. The CFU-GM, BFU-E and CFU-MEG in the B18 mice were similar to the B-gal control (Table II).

Administration of the adenovirus encoding B18 resulted in an increase in CFU-GM (myeloid) and BFU-E (erythroid) progenitors in the spleen compared to animals that received the B-gal virus on day 7. The increase in progenitors in the B18 mice was 11-fold in CFU-GM and a 52-fold in BFU-E (Table II). There was a 2-fold increase in CFU-MEG at day 7 for the B18 mice. By day 21 no significant differences were observed in splenic CFU-MEG or BFU-E between the groups (Table II). A 3-fold decrease in CFU-GM was observed in mice that received adenovirus encoding B18. A slight increase in spleen size at day 7 was observed in the B18 group. This is consistent with an increase in splenic cellularity. By day 14 and day 21 spleen weights were similar to the control group (Table III). Macroscopic analysis of the spleen showed an increase in splenic extramedullary hematopoiesis of the B18 mice on day 7 compared to the control.

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The bone marrow myeloid: erythroid ratios (Table IV) suggest a granulocytic hyperplasia with a possible erythroid hypoplasia in mice that received adenovirus B18 on day 7. By day 21 the ratio in the B-gal group was higher. No changes were observed in the IL6 serum levels.

These results show a hematopoietic activity associated with the administration of adenovirus encoding B18 (human CTLA-8). Increases in neutrophils and white blood cells were observed at day 7 in animals that received B18 adenovirus. The data showed that B18 resulted in increase in splenic CFU-GM and BFU-E 7 days post administration compared to the control animals. Splenic extramedullary hematopoiesis on day 7 support that B18 exhibits a hematopoietic growth activity. These data suggest that B18 may moblize early hematopoietic precursors.

Table I: Peripheral hematology for day 2, 5, 7, 10, 14, and 21.

AVC 17.1 32.0 2.10 52.4 2.21 5.5 4.6 2.21 5.5 5.6 1.21 5.5 1.2 5.6 1.2 5.7 5.7 5.8 5.8 1.1.8 1.2.5 0.45 1.2.	Group A West Month AMC West The Year The	B-Gal #1 B-Gal #2 B-Gal #3 B-Gal #4 AVG	WBC x10^3/uL	Noute % ±	ANC 1003/ut	Lymphs	ALC	For	Monos	RBC I						
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SERIE 10.7	STEAL 1.7				2.19				7.8	11.62						
Single 11-5	Size 11-4 53 67.75 67.2			4.6	0.19		0.61		1.4						14.92	185.31
Single S. S. S. S. S. S. S. S	1918 4						3.53					0.40	48.0	632	10.90	68.89
State Stat	Sign S.						2.00	ŏ	9	11,16			51.2			
Sept	Part 14 14 15 16 17 17 17 17 17 17 17	B18 #4	6.4			55								904		
Second Company Compa	No.							0.7	1.3						1,55	27.57
Compage Will	Company WINC March AMC Lympha ALC East Moone REC. Rect.	SEM IA DA ME IN														
Company Tribuy	Second 19		B18 (Platel	ots) [Day 54-2		ALC: T	Foe	Monos	RBC I	Retics /	bs Retics	нст	PLT	RPI	Abs RPII
BOAIR 1.08 10.8 20.2 11 1.06 70 5.33 3 5 1.02 5.31 5.05 6.24 6.4 10.4 10.4 10.8 10.	Fig. 12			Neura :	410^3/UL	* x				x10^6/uL	%	x10^6/uL	*			
B-Old #2 10.5 20. 2.11 76 20.7 2 11 11.12 3.00 0.38 51.2 515 52.5 87.40	B-Gard 10.6 10.9 10.1 10.1 10.1 10.1 10.1 10.1 10.1	B-Gal #1	7.6													
Sept	Second Column Second C			20						10.72	3.40					
Second Column C	Sept 0.8 5.5 2.05 70.4 6.61 1.5 5.7 6.61 1.5 5.7 6.61 1.5								4	10.22	6.21	0.63	47.0			152.12
Big 14.2 17 2.66 71 10.51 1 10 12.66 2.11 2.26 2.11 2.12 12.0 2.12 12.0 1	Bill 12		9.5		2.20	70.8			5.3					1021		
Simple 14.9	Display 14.2 17							0.6	10 21					1204		91.14
Bill 84 12.8 30 3.84 59 7.55 10 12.12 4.12 4.12 4.13 5.00 11.05 11.0	Bill 81 12.0 30 3.44 59 7.55 1 10 12.12 3.22 0.33 2.05 1.05 11.25 12.25			18			7.53	ż	8 Ì	9.80	3.32		44.6		14.33	127.25
Bit 84	Sign		12.8	30	3.84	59	7.55	1		12.12	4.12					
Story AS-I-G. Bits (Priestedist)	SEMP AND PROFESSION A	B18 #4	16.0		9.28										11,95	129.28
Shery AS-4-8. B16 [Pietelett)	Sheet AS-1				144				1.2	0.63						19.51
Charles Char	Check		<u> </u>		1933											
Group C	Group A Vision No. Xi Yi Yi Xi Xi Xi Xi Xi						ALC I	Eos I	Monos I	RBC I	Retice	Abs Retics I				Abs RPt
B-Gul #1 152 140 12 168 81 1131 0 7 11130 3.54 0.39 852 1426 107.24 16.8	Bods 152	Group C		***	210*3A/L	1 % x	וועביי סור		*	x10^6A/L	%	x10^6/uL	%		- %	
B-Gal 87 14.0 12 1.68 15 11.30 0 12 1.00 12 1.00 12 1.00 0.59 4.00 15.5	B-Call #7 14.0 12 1.68 87 10.30 0 7 12 10.20 2.42 0.52 4.43 10.20 77 10.20 2.42 0.52 4.44 10.20 10.20 11.20 10.20 11.20 10.20 11.20 10.20 11.20 10.20 11.20 10.20 11.20 10.20 11.20 10.20 11.20 10.20 11.20 10.20 11.20 10.20 11.20 10.20 11.20 10.20 11.20 10.20 11.20 10.20 11.20 10.20 11.20	B-Gal #1	15.2	14	2.13	69	10.49					0.39	50.8			
SEM 0.4 0.7 0.14 33 0.40 62 12.03 0 0 5 10.14 0.58 0.05 0.0 771 12.01 11.51 0.58 0.05 0.05 0.05 0.0 771 12.03 11.51 0.51 0.05 0.05 0.05 0.05 0.05 0.0	Sept 14.7 12.3 1.86 74.3 10.88 0.7 11.7 11.11 14.87 12.50 11.87 14.51 14.87 14.8						11.34							952	11.49	109.38
SEM	SEM O.A O.7 O.14 3.5 O.25 O.3 2.6 O.14 O.58 O.05 O.3 O.7 O.16 O.5 O.3 O.5 O.3 O.5 O.3 O.5 O.3 O.5		14.8	14133			10.88			11.11			51.0			127.13
Bit 82 25.4 39 8.0 62 12.46 0 8 8.46 6.25 6.27 7.15 11.07	Bit 19.4 33 8.40 65 14.66 0 8 0.45 60.5 60.57 42.6 12.65 12.65 16.65 1	SEM	0.4	0.7		3.5	0.25			0.14						110 59
Big 84 12.8 15 13.2 75 9.0 0 0 6 9.74 6.17 0.50 44.4 1076 15.41 165.81 188.8 180.40 189.84 12.8 15 13.2 75 9.0 0 10 9.54 6.26 0.60 43.4 1136 15.88 180.40 189.84 12.8 15 13.2	## 18 64 22.5 24 10.38 EO 11.00 0 6 9.74 5.17 0.50 44.4 1076 15.41 16.85 18.86 12.8 15 1.92 75 9.50 0 0 7.2 8.72 5.10 0.49 43.4 11.36 15.88 15.40 6.82 2.8 6.3 1.96 6.56 0.80 11.72 0.0 7.2 8.72 5.10 0.49 44.2 1091 44.15 15.44 6.82 2.8 6.3 1.96 6.56 0.80 0.0 1.1 0.15 0.77 0.44 28 0.97 0.47 28 0.97 0.47 28 0.97 0.47 28 0.97 0.47 28 0.97 1.1 0.15 0.15 0.15 0.77 0.47 28 0.97 0.47 28 0.97 1.1 0.15							, l		946		457				160.87
B18 84 12.8 15 1.92 75 9.50 0 12 9.54 62.8 0.60 43.4 11.96 13.06 14.15 15.04 15.06 1.172 0.07 1.1 0.15 0.76 0.67 0.4 28.0 0.47 15. 15.04 15.	Big 44 12.6 15. 1.52 75 9.60 0.0 17.2 0.0 17.2 0.075 0.40 44.2 19.6 19.6 0.87 15.5 Big 4 2.8 6.3 1.96 5.6 0.80 0.0 1.17 0.15 0.76 0.47 0.4 19.6 0.87 15.5 Big 5 2.8 6.3 1.96 1.9								8	9.74	5,17	0.50	44.4			
Study AS4-48. B16 (Pistelets) Day 105-5-96. Day 10. Da	AVG 20.3 37.3 (1.13 0.5 0.5 0.0 1.11 0.15 0.75 0.07 0.4 88 0.87 15.1 (Study ASA-48_B) 116 (Pintelett)			15	1.92	75			. 19							
Study AS-4-(8. Bits (Pitriefets) Day 105-3-96. Day 145-7-96. Day 14. St. C Day 1	Study ASS-4.8 316 (Pithelets)				7.15				1.1					88		15.19
Group A Vigo Newts ANC Lymphs ALC Ess Mones RBC Relics Ass Redics HCT -PLT MPR ADDRESS ASS Redices	Group A ViGC Notate ANC Lymphe ALC East Monos RBC Monos RBC Abs Relics HCT FLT RFR Abs Relics HCT FLT HCT RFR Abs Relics HCT FLT HCT RFR Abs Relics HCT FLT HCT RFR Abs Relics HCT FLT R	SEH-	2.8	6.3	1.30	· · · ·										
Group A Wilco Newtra Art. St. 170-20t. St.	Group A VISC New Trip*SML No. No				Day 105			F	1 11		Detlan	Aba Batica I	нст	I PIT I	APH	Abs RPI
B-Call #1 18.6 17 3.16 69 12.83 3 11 10.24 10.40	B-Gal #1 18.5 17 3.16 69 12.83 3 11 10.22 12.41 127 49.8 14.02 12.43 B-Gal #2 13.2 18 2.11 79 10.04 1 1 4 10.04 6.00 0.53 48.4 1229 14.02 12.43 B-Gal #3 19.5 16 3.14 74 14.50 0 10 10.72 6.25 0.67 49.4 1339 14.05 12.13 B-Gal #3 19.5 16 3.14 74 14.50 0 10 10.72 6.25 0.67 49.4 1339 14.05 12.13 B-Gal #4 18.8 21 17.5 3.08 7.72 13.39 3 4 7.3 10.47 8.06 0.41 4.4 12.9 10.5 12.5 12.5 12.5 12.5 12.5 12.5 12.5 12	Group A			AHC V1042Ad	Lympna				X10~6VrL	%	X10^6ArL	*	X10^3/L	%	x10 -344L
B-Gail #2 13.2 18 2.11 79 10.43 1 10.72 8.25 0.57 49.4 13.08 16.5 16.3 14 74 14.50 0 10.72 8.25 0.57 49.4 13.08 14.5 15.08 14.2 15.3 15	B-Gal #2 132 18 2.11 79 10.43 1 4 10.47 259 0.57 49.4 1239 16.50 221.84 B-Gal #4 18.8 19.5 16 5.14 74 14.50 0 10 10.72 62.5 0.57 49.4 1239 16.50 124.35 15.326 B-Gal #4 18.8 21 3.91 72 13.59 3 4 10.44 7.59 0.57 49.4 10.24 10.24 10.25 15.326 SEM 17.5 1.2 0.37 2.1 0.86 0.5 19.5 0.10 19.7 10.47 2.06 0.84 48.4 10.24 10.25 15.326 SEM 17.5 1.2 0.37 2.1 0.86 0.5 19.5 0.10 11.87 10.41 2.06 0.8 19.1 10.25 15.326 18.50 19.5 19.5 19.5 19.5 19.5 19.5 19.5 19.5	B-Gal 61			3.16	69	12.83	3 .		10.22	12.41	1.27	46.8		16.20	
B-Gal #4 18.8 21 17.5 17.5 13.30 3 4 10.44 7.59 0.79 44.4 16.88 14.35 153.28 15.80 15.80 17.5 17.5 17.5 13.5 12.	B-Gail #1 18.8	B-Gal #2			211	79					8.00		49.4		18.58	
SEM 17.5 17.5 30.8 77.5 12.79 1.8 7.3 10.77 8.06 0.81 12.49 15.40 15.50 15.0 15 0.6 91 10.58 2.0 18.0 18.0 11.2 0.37 2.1 0.56 0.8 1.9 0.10 11.97 1.04 92.1 15.0 15.0 15.0 15.0 15.0 15.0 15.0 15	See 1.7.5 17.5 13.8 77.5 12.7 13.1		19.5			1 /2	13.39		4		7.59	0.79	48.4-	1068	14.35	
SEM 1.5 1.2 0.57 2.1 0.86 0.8 1.9 0.10 11.97 1.0.4 39.2 1750 14.49 25.500	SEM				3.08	73.5	12.79								15.40	193.74
Bill 87 14.2 35 6.16 57 10.00 7 9.00 8.48 0.55 62.0 1104 18.58 208.44 Bill 87 16.2 39 6.32 57 9.23 1 3 4.74 16.77 0.79 22.4 6.94 6.94 Bill 87 16.2 39 6.32 57 9.23 1 8 9.30 9.30 9.30 9.20 42.0 1416 16.81 239.0 Bill 84 14.2 2.5 3.55 66 9.37 1 8 9.30 9.30 9.30 9.30 42.0 1416 16.81 239.0 See	Bit 8 14.2 33 6.16 57 10.03 1 7 8.04 8.48 0.86 42.0 1104 18.88 200.4 Bit 8 2 17.6 33 6.18 57 10.03 1 7 8.04 8.48 0.86 42.0 1104 18.88 200.4 Bit 8 2 16.2 39 6.32 35.5 66 9.77 1 8 9.30 9.93 0.92 42.0 1416 16.81 230.3 AVG 15.5 33.0 6.18 69.0 8.15 2.0 6.0 75.3 12.04 6.90 36.4 129.4 19.44 240.4 SEM 0.8 2.9 0.86 2.3 0.43 1.0 1.1 1.08 1.57 0.05 4.7 189 3.24 11.5 Study A54-4B_B16 (Pintelets) Dsy 14_5-7-65.	SEM	1 1.5	1.2		2.1									14.49	
Bill 873 16.2 39 63.2 57 92.3 1 3 4.74 16.77 0.79 22.4 89.4 29.1	Bill 843 16.2 79 6.32 57 9.23 1 3 4.74 16.77 0.79 22.4 894 29.10 283.05 84 14.2 25 3.55 66 9.77 1 8 9.30 9.30 0.92 42.0 1416 16.81 238.07 17.0 18.5 12.0 6.0 7.95 12.0 0.95 4.7 189 3.24 11.5 11.1 1.08 1.07 0.95 4.7 189 3.24 11.5 11.1 1.08 1.07 0.95 4.7 189 3.24 11.5 11.1 1.08 1.07 0.95 4.7 189 3.24 11.5 11.1 1.08 1.07 0.95 4.7 189 3.24 11.5 1.07 0.95 4.7 189 3.24 11.5 1.07 0.95 4.7 189 3.24 11.5 1.07 0.95 4.7 189 3.24 11.5 1.07 0.95 4.7 189 3.24 11.5 1.07 0.95 4.7 189 3.24 11.5 1.07 0.95 4.7 1.092 7.07 0.77 0.75 50.8 136.0 11.03 15.01 1.09 0.96 0.95 0.	818#1						•	7	9.04	9.48	0.86	42.0		18.58	208.44
B18 #4 14.2 25 3.5.5 66 9.77 1 8 6.0 7.95 12.04 6.90 32.4 11294 11.95 12.04 6.90 32.4 11.95 12.04 6.90 32.4 11.95 12.04 6.90 32.4 11.95 12.04 6.90 32.4 11.95 12.04 6.90 32.4 11.95 12.04 6.90 32.4 11.95 12.04 6.90 32.4 11.95 12.04	Bill 84		16.2		6.32	57	9.23	1		4.74						
Study AS4-4B_B[5] (Platelets) Osy 14_57-96.	AVG 18.1 21.8 4.03 71.8 12.2 0.8 12.2 0.8 12.2 0.8 12.2 12.2 13.5	B18 #4	14.2				9.37	1		9.30						240.61
Study A54-48_B18 (Pietelets)	Study ASA-4B_BIS (Platelets) OBY 14_5-7-66,						0.43		1.1	1.08	1.57	0.05	4.7	189		11.77
Group B WBC Neutra ANC Lympha ALC Eos Monos RBC Neutra Anc A	Group B WBC Heuts ANC Lymphs ALC Eos Monos RBC RBCS ASS RBCS ANC CT CT	SEM														
Group 6 Tri0-Stut % Xri0-Stut % Xr	Group No. St. No. S	Study A54-4B.		elets)	Day 14	5-7-96,	ALC:	En-	Monne	RAC	Retica	Abs Retics	. HCT	PLY	RPK	Abs RPII
B-Gal #1 17.9 18 3.20 74 13.17 0 8 10.92 7.07 0.77 50.8 1360 11.03 15.01 B-Gal #2 20.4 26 5.30 66 13.46 1 7 10.92 7.07 0.77 50.8 1360 11.03 150.1 B-Gal #3 16.0 7 1.12 90 14.40 1 3 11.38 6.41 0.73 52.8 1298 7.36 95.5 B-Gal #4 18.0 36 6.48 57 10.26 1 6 9.30 7.52 0.71 43.0 1672 10.05 168.04 AVG 18.1 21.8 4.03 71.5 12.82 0.8 6.0 10.51 6.77 0.71 43.4 1487 9.15 13.4 BEEM 0.9 6.1 1.18 7.0 0.89 0.2 1.1 0.45 6.77 0.71 43.4 1487 9.15 13.4 B18 #2 15.4 31 4.77 58 8.93 2 9 9.76 10.33 10.11 44.6 10.92 41.29 156.05 B18 #3 13.4 42 5.53 39 5.23 0 19 10.34 4.99 0.52 46.8 1376 15.79 217.27 B18 #4 11.6 57 6.61 34 3.94 2 7 9.30 5.57 0.52 48.6 1376 15.79 217.27 B18 #4 11.6 57 6.61 34 3.94 2 7 9.30 5.56 0.66 6.65 6.66 6.55 1206 14.06 181.3 SEM 0.9 10.1 1.14 10.7 1.93 0.5 2.7 0.28 1.23 0.12 1.1 70 1.59 20 Study A54-48. B18 (Pistolets) Day 21. 5.1498	B-Gal #1 17.8 18 3.20 74 13.17 0 8 10.92 7.07 0.77 50.8 1360 11.03 150.01 B-Gal #2 20.4 26 5.30 66 13.46 1 7 10.92 7.07 0.77 50.8 1616 8.16 132.19 B-Gal #2 20.4 26 5.30 66 13.46 1 7 10.92 7.07 0.77 50.8 1616 8.16 132.19 B-Gal #4 18.0 7 1.12 90 14.40 1 3 11.36 6.41 0.73 52.8 1298 7.36 95.53 B-Gal #4 18.0 35 6.46 57 10.28 1 6 9.30 7.52 0.71 43.0 1672 10.05 168.0 AVG 18.1 15.4 9 1.39 81 12.47 1 9 10.65 5.74 0.61 46.2 1262 9.51 120.02 B18 #1 15.4 31 4.77 58 8.93 2 9 9.76 10.33 1.01 44.6 1092 14.29 156.05 B18 #2 13.4 42 5.53 39 5.23 0 19 10.34 4.99 0.52 43.0 10.92 16.65 181.33 B18 #4 11.5 57 6.61 34 3.94 2 7 9.38 8.57 0.52 43.0 10.92 16.65 181.33 B4 W 14.0 57 6.61 34 3.94 2 7 9.38 8.57 0.52 43.0 10.92 16.65 181.33 B4 W 14.0 54 4.65 53.0 7.64 1.3 11.0 10.03 6.66 0.56 45.5 1206 14.06 16.8 AVG 14.0 54 4.65 53.0 7.64 1.3 11.0 10.03 6.66 0.56 45.5 1206 14.06 16.8 AVG 14.0 54 4.65 53.0 7.64 1.3 11.0 10.03 6.66 0.56 45.5 1206 14.06 16.8 AVG 14.0 54 4.65 53.0 7.64 1.3 11.0 10.03 6.66 0.56 45.5 1206 14.06 16.8 AVG 14.0 54 4.65 53.0 7.64 1.3 13.0 10.03 6.66 0.56 45.5 1206 14.06 16.8 B4 11 13 13 13 13 13 13 1	Croup B		Meuts 4		. %	11/E* 01x	%	%	x10^6/dL	. %	x10^6A/L	×			x10 ^3//L
B-Gal 92 20.4 26 5.30 66 13.40 1 3 11.25 6.41 0.77 52.8 12.90 7.36 65.53	B-Call #2 20.4 26 5.30 66 13.48 1 3 11.32 6.41 0.73 32.8 12.99 7.26 95.53	B-Gal #1			3.20	74					5.97	0.65				
B-Gal #1 16.0 56.48 57 10.26 1 6 9.30 7.62 0.77 0.71 43.0 1672 10.05 168.04	B-Gal #1 18.0	B-Gai #2													7,36	95.53
AVG	SEM O.9	B-Gal #3	16.0			57	10,26	i	6	9.30	7.62	0.71	43.0	1672	10,05	
SEM Q.9 6.1 1.18 7.0 0.89 0.3 1.1 0.48 0.32 0.51 2.2 2.5 0.51 120.00	BIS 61 15.4 9 1.39 81 12.47 1 9 10.62 5.74 0.81 45.2 1263 9.51 120.02 BIS 82 15.4 31 4.77 58 8.93 2 9 9.76 10.33 1.01 44.6 10.92 14.29 158.05 BIS 82 15.4 31 4.77 58 8.93 2 9 9.76 10.33 1.01 44.6 10.92 14.29 158.05 BIS 83 13.4 42 5.53 39 5.23 0 19 10.34 4.99 0.52 44.8 1376 15.79 217.37 BIS 84 11.5 57 6.51 34 3.94 2 7 9.38 8.57 0.52 44.0 10.92 16.56 181.3 AVG 14.0 34.8 4.50 53.0 7.54 1.3 11.0 10.03 6.65 0.56 45.5 1206 14.06 168. AVG 4.0 50.0 50.0 1.14 10.7 1.93 0.5 2.7 0.26 1.23 0.12 1.1 70 1.59 20. Study A54-48_BIS (Pistolets) Day 21.5-14-96. Group A WBC Neuris ANC Lymphs ALC E0.8 Monos RBC Retice ADE Retice HCT PLT RPR ADE RPS ADE RETICE ADERICAN ADE RETICE ADERICAN ADERICA	AVC		1 21.6	4.01	71.8	12.82		6.0				49.4			136.4 15.4
Bill #1 15.4 9 1.39 81 1.47 58 8.83 2 9 9.76 10.21 1.01 44.6 10.02 14.25 156.05 Bill #2 15.4 42 5.63 39 5.21 0 19 10.34 4.99 0.52 48.8 1376 15.79 217.27 Bill #3 13.4 42 5.63 39 5.21 0 19 10.34 4.99 0.52 48.8 1376 15.79 217.27 Bill #4 11.6 57 6.61 34 3.94 2 7 9.38 5.77 0.25 43.0 10.92 16.56 181.21 Bill #4 11.6 57 6.61 34 3.94 2 7 9.38 5.77 0.25 43.0 10.92 16.56 181.21 Bill #4 11.6 57 6.61 34 3.94	Bill 8/1 15.4 9 1.39 01 12.7 7 58 8.93 2 9 9.75 10.23 1.01 1.01 1.02 14.15 10.02 14.29 15.6.5	SEL	u <u>0.</u>	6.1	1.10			1 0.3	 1.1	1062			48.2			120.02
Bib 8/2 13.4 42 5.63 39 5.21 0 19 10.34 4.99 0.52 48.6 1378 15.79 217.27 Bib 8/3 13.4 42 5.63 34 3.94 2 7 9.38 5.57 0.52 43.0 1092 16.65 191.92 Bib 8/4 11.6 57 6.61 34 3.94 2 7 9.38 5.57 0.52 43.0 1092 16.65 191.92 AVG	Bit						8.93	اغا		9.76	10.33	1.01	44.6	1092		
BiB 84 11.6 57 6.61 34 3.94 2 7 7 3.95 3.94 2 7 7 3.95	Big 84			42	5.63	39	5.23	0	19	10.34	4,99	0.52				217.27
AVG 14.0 34.8 4.60 53.0 7.64 1.3 11.0 0.5 2.7 0.28 1.23 0.12 11.1 70 1.59 20 5EM 0.9 10.1 1.14 10.7 1.93 0.5 2.7 0.28 1.23 0.12 11.1 70 1.59 20 5EM 1.23 0.12 11.1 70 1.25 1.68	AVG 14.0 34.8 4.50 53.0 7.58 1.3 11.0 1.50 52.0 5.50 6.12 1.1 70 1.59 20. Study AS4-4B. B18 (Pinteleds) Day 21.5-14-96. Study AS4-4B. B18 (Pinteleds) Day 21.5-14-96. Group A WGC Nexts ANC Lymphs ALC Eos Monos RBC X 10-50.1	B18 #4	11.6	57									43.0			168.8
Study A54-4B_B 18 (Platfolds) Day 21_5-14-96. Eos Monos RBC Retics Abs Retics HCT PLT RPH Abs RETICS Retics Abs Retics RCT PLT RPH Abs RETICS Retics Abs Retics	Study AS-4-4B B18 (Pirtolets) Day 21.5-14-96. Eos Monos RBC Retics Abs Retics HCT PLT RPR Abs RPG Abs Retics HCT PLT RPR Abs RPG Retics Reti	AVO		94.5						0.20	1.23				1.59	20.5
Group A WBC Nexts ANC Lymphs ALC Eos Monos RBC Retics Abs Ret	Coroup A WBC Nexts ANC Lymphs ALC Eos Monos RBC Retics AB retics RC Fr. To Aut		**1													
Croup A WBC Macris Anti-Sult % x10 *Sult % x 110 *Su	Group A WBC	Study A54-4B	_B18 (Pla	lolots)	Day 21		A1.0	For	Monoe	RRC	Retice	Abs Retice	HCT			Abs RPR
B-Gal #1 25.4 23 5.84 67 17.02 0 10 9.22 8.15 0.75 42.8 1776 9.61 170.55 B-Gal #1 25.4 23 5.84 67 17.02 0 12 9.50 9.95 0.95 44.4 1662 9.44 156.2 B-Gal #2 19.6 19 3.72 69 13.52 0 12 9.50 9.95 0.95 44.4 1662 9.44 156.2 B-Gal #2 27.8 11 3.04 82 22.63 3 4 9.74 8.84 0.86 45.8 1584 11.45 192.85 B-Gal #2 28.0 13 3.84 83 22.24 0 4 9.74 8.84 0.86 45.8 1584 11.45 192.85 B-Gal #2 28.0 13 3.84 83 22.24 0 4 9.74 8.84 0.86 45.8 1584 11.45 192.85 B-Gal #2 28.0 15 5.5 4.06 75.3 19.10 0.8 7.5 9.38 8.62 0.81 43.7 1617 10.25 188 189.6	B-Gal 41 25.4 23 6.84 67 17.02 0 10 9.22 6.15 0.75 42.8 1776 9.61 170.67	Group A	WBC	Monte	MNC MICASH							x10^6/uL	%	x10^3/ut.	×	×10 ^3//L
B-Gal #2 19.6 19 3.72 69 13.52 0 12 9.50 9.55 0.59 45.8 1654 11.45 132.56 B-Gal #3 27.8 11 3.04 82 22.63 3 4 9.74 8.84 0.56 45.8 1654 11.45 132.56 B-Gal #4 28.0 13 5.84 83 22.24 0 4 9.74 8.84 0.56 41.6 13.46 10.48 141.05 B-Gal #4 28.0 13 5.84 83 22.24 0 4 9.74 8.84 0.58 41.6 13.46 10.48 141.05 B-Gal #4 28.0 15 5.84 83 22.24 0 4 9.74 8.85 0.58 41.6 13.46 10.48 141.05 B-Gal #4 28.0 15 5.84 83 22.24 0 8.87 13.74 1617 10.25 18.65 18	D-Gal #2 19.6 19 3.72 69 13.52 0 12 9.50 9.95 0.85 45.8 1682 11.45 192.62 B-Gal #3 27.8 11 3.04 82 22.63 3 4 9.74 8.84 0.85 45.8 1684 11.45 192.62 B-Gal #4 28.0 13 3.84 83 22.24 0 4 9.04 7.54 0.68 41.6 13.6 10.48 141.05 AVG 25.2 16.5 4.06 75.3 19.10 0.8 7.5 9.38 8.62 0.81 43.7 1617 10.25 18.8 B-Gal #4 28.0 15.5 4.06 4.2 2.33 0.8 2.1 0.15 0.52 0.06 0.9 94 0.45 10.8 B-Gal #4 10.6 9 1.67 63 15.44 1 7 9.84 7.40 0.73 43.8 1642 63.7 137.44 B-Gal #4 10.6 9 1.67 63 15.44 1 7 9.84 7.40 0.73 43.8 1642 63.7 137.44 B-Gal #4 15.2 16.2 48 7.78 45 7.29 1 6 11.10 7.97 0.88 51.4 1970 10.78 212.37 B-Gal #4 15.2 18 2.74 74 11.25 2 6 9.84 7.02 0.68 43.0 1422 7.60 106.07 B-Gal #4 15.2 18 2.74 74 11.25 2 6 9.84 7.02 0.68 43.0 1422 7.60 106.07 B-Gal #4 15.2 18 2.74 74 11.25 2 6 9.84 7.02 0.68 43.0 1422 7.60 106.07 B-Gal #4 15.2 18 2.74 74 11.25 2 6 9.84 7.02 0.68 43.0 1422 7.60 106.07 B-Gal #4 15.2 18 2.74 74 11.25 2 6 9.84 7.02 0.68 43.0 1422 7.60 106.07 B-Gal #4 15.2 18 2.74 74 11.25 2 6 9.84 7.02 0.68 43.0 1422 7.60 106.07 B-Gal #4 15.2 18 2.74 74 11.25 2 6 9.53 10.60 0.95 44.1 1551 9.31 1551	B-Gal at		23	5.84	67	17.02	0								
B-Gal #3 27.8 11 3.04 82 22.3 0 4 9.04 7.54 0.58 41.6 13.46 10.48 141.05 B-Gal #4 28.0 13 3.54 83 23.24 0 4 9.04 7.54 0.58 41.6 13.46 10.48 141.05 AVG 25.2 16.5 4.06 75.3 19.10 0.8 7.5 9.38 8.52 0.81 43.7 1617 10.25 185 SEM 1.9 2.8 0.61 4.2 2.33 0.8 2.1 0.15 0.52 0.08 0.9 94 0.46 11.	B-Gal #3 27.6 11 3.04 83 22.24 0 4 9.04 7.54 0.68 41.6 10.48 141.06 8.44 28.0 15 3.84 83 232.4 0 8.75 9.38 8.52 0.51 43.7 1617 10.25 183 18.6 18.6 18.6 18.6 18.6 18.6 18.6 18.6	B-Gal #2	19.6	19												192.82
AVG 25.2 16.5 4.06 75.3 19.10 0.8 7.5 9.38 8.52 0.81 43.7 1617 10.25 185 SEM 1.9 2.8 0.61 4.2 2.33 0.8 2.1 0.15 0.52 0.06 0.9 94 0.46 10 0.45	AVG 25.2 16.5 4.06 75.3 19.10 0.8 7.5 9.38 4.62 0.81 43.7 1617 10.25 165. SEM 1.9 2.8 0.61 4.2 2.33 0.8 2.1 0.15 0.52 0.06 0.9 9.4 0.46 10. B16 #1 18.6 9 1.67 83 15.44 1 7 9.96 7.00 0.73 43.8 1642 8.37 137.44 B16 #2 16.2 48 7.78 45 7.29 1 6 11.10 7.97 0.88 51.4 1970 10.78 2123. B18 #3 14.8 53 7.84 42 6.22 0 5 7.52 20.26 1.52 30.0 1568 10.50 164.64 B18 #3 14.8 53 7.84 42 6.22 0 6 9.84 7.00 0.73 43.8 1642 8.37 137.44 B18 #4 15.2 18 2.74 74 1.125 2 6 9.84 7.00 0.88 43.0 1452 7.60 10.01	P.C1 #1	27.6							9.04		0.68	41.6	1346	10.48	141.06
SEM 19 2.6 0.61 4.2 2.33 0.8 2.1 0.15 0.52 0.06 0.9 94 0.70 177.4	SEM 1,0 2.8 0.51 4.2 2.33 0.8 2.1 0.15 0.52 0.08 0.8 0.8 142 0.25 0.07 17.44 0.72 0.88 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.					6 75.3	19.10	0.1	8 7.5	9.38	8.62	0.81	43.			165.3
	B18 #1 18.6 9 1.67 83 13.44 1 7.97 7.97 7.97 7.98 51.4 1970 10.78 212.37 10.88 18.8 21.8 2	B-Gal #4			8 0.6	1 4.2		0.0	8 2				43.0	91 94		
818 #1 18.6 # 779 45 729 1 6 11.10 7.97 O.88 51.4 1970 10.78 212.37	818 #2 16.2 5.3 7.84 42 8.22 0 5 7.32 20.26 1.52 38.0 1568 10.50 164.84 818 #3 14.8 53 7.84 42 8.22 0 5 9.84 7.02 0.68 43.0 1422 7.60 108.0 818 #4 15.2 18 2.74 74 11.25 2 6 9.84 7.02 0.68 43.0 1422 7.60 108.0 43.0 14.2 14.2 14.2 14.2 14.2 14.2 14.2 14.2	B-Gal #4 AV		. 1 - 0	1.67			1 .	6							
818 #2 162 43 784 47 622 0 5 7.52 2026 1.52 38.0 1568 10.50 164.6	B18 #4 15.2 18 2.74 74 11.25 2 6 9.84 7.02 0.68 43.0 14.22 7.60 108.07 818 #4 15.2 18 2.74 74 11.25 2 6 9.84 7.02 0.68 43.0 14.22 7.60 108.07 818 #4 15.2 18 2.74 74 11.25 2 6 9.84 7.02 0.68 43.0 14.22 7.60 108.07 818 #4 15.2 18 2.74 74 11.25 2 6 9.84 7.02 0.68 43.0 14.22 7.60 108.07 818 #4 15.2 18 2.74 74 11.25 2 6 9.84 7.02 0.68 43.0 14.22 7.60 108.07 818 #4 15.2 18 2.74 74 11.25 2 6 9.84 7.02 0.68 43.0 14.22 7.60 108.07 818 #4 15.2 18 2.74 74 11.25 2 6 9.84 7.02 0.68 43.0 14.22 7.60 108.07 818 #4 15.2 18 2.74 74 11.25 2 6 9.84 7.02 0.68 43.0 14.22 7.60 108.07 818 #4 15.2 18 2.74 74 11.25 2 7.60 108.07 818 #4 15.2 18 2.74 74 11.25 2 7.60 108.07 818 #4 15.2 18 2.74 818 #4 15.2 18 2.74 818 #4 18.2 18 2.74 818 818 818 818 818 818 818 818 818 81	B-Gal #4 AV SEI B18 #1	18.6			1 46										
B18 63 14.5 18 2.74 74 11.25 2 6 9.84 7.02 0.68 43.0 1422 7.60 108.07	AVG 16.2 32.0 5.01 61.0 10.05 1.0 6.0 9.53 10.66 0.55 44.1 1651 9.31 13.0	B-Gai #4 AV SEI B18 #1 B18 #2	18.6 16.2	48	7.78	45			5			1.52	38.0	1568	10.50	164.64
AVG 16.2 32.0 5.01 - 61.0 10.05 1.0 6.0 9.53 10.66 0.95 44.1 1651 9.31	1 equi) not 169 1.61(10.3 2.10) 0.4(0.4) 0.4(3.2) 0.40(2.0) 1(0) 0.70	B-Gal #4 AV SE B18 #1 B18 #2 B18 #3	18.6 16.2 14.8	48 53	7.78 7.84	42 74	6.22 11.25	2	6	7.52 9.64	20.26 7.02	1.52 0.68	38.0 43.0	1568 1422	10.50 7.60	108.07
	3EM V.3.1 10.0	B-Gal #4 AV SE B16 #1 B10 #2 B18 #3 B18 #4	18.6 16.2 14.8 15.2 (G 16	48 53 18 2 32	7,78 7,84 2,74 0 5.0	42 74 11 · 61.0	6.22 11.25 10.05	0 2 1.	0 6.	7.52 9.84 0 9.5	20.26 7.02 10.66	1.52 0.68 0.95	38.0 43.0 44.	1568 1422 1 1651	10.50 7.60 9.31	

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Table II: Bone marrow and Splenic Hematopoietic Progenitors

	CFU	-MEG	CFU-GM		BFU-E			
Bone Marrow*	B-Gal	B18	B-Gal	B18	B-Gal	B18		
Day 7	16.0 ±	15.7 ± 3.1	307 ±117	241±78	51 ± 19	25 ± 11		
Day 14	10.7 ± 2.3	15.3 ± 1.2	233 ± 15	373±35	30 ± 10	60 ± 30		
Day 21	5.7 ± 0.6	6.7 ± 3.1	170 ± 17	160±27	40 ± 10	27 ± 6		
Spleen**								
Day 7	9.3 ± 1.6	19.5 ± 1.5	27 ± 3	298 ± 6	1.3 ± 1.2	68 ± 10		
Day 14	9.7 ± 0.6	12.7 ± 0.6	267 ± 32	197 ±21	33 ± 6	10 ± 10		
Day 21	17.0 ± 1.0	19.3 ± 2.5	187 ± 6	73 ± 15	23 ± 6 .	23 ± 6		

Hematopoietic precursors were determined form pooled spleen and bone marrow samples from four animals in each group. For quantitation of CFU-GM and BFU-E, either 1 x 10⁴ bone marrow cells or 1 x 10⁵ spleen cells were added to complete alpha methylcelluose medium (0.9% methylcellulose in alpha medium, 30% fetal bovine serum, 1% bovine serum albumin, 10-4M 2-mercaptoethanol, 2 mM L-glutamine, 2% murine spleen cell conditioned medium, and 3 U/mL erythropoietin) and aliquoted into 35 mm tissue culture dishes in a final volume of 1.0 mL. Cultures were incubated for 7 days at 37°C, and 5% CO₂. Microscopic colonies were defined as clusters of 50 or more cells. For quantitaion of CFU-MEG, either 1 x 10⁵ bone marrow cells or 1 x 10⁶ spleen cells were added to complete alpha methylcellulose medium and incubated as described above. Megakaryocyte colonies were defined as a group of 3 or more cells.

^{*}Bone marrow progenitors are represented as mean ± sd number of colonies per 10⁵ cells.

^{**}Spleen progenitors are represented as mean ± sd number of colonies per 10⁶ cells.

Table III: Spleen Weights and Femur Cellularity

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Spleen Wt.	B-Gal	B18		Femur	B-Gal	B18
(Mg)				Cellularity		
				$(x10^6)$		
Day 7	187 ± 19	224 ± 29		Day 7	28	23
Day 14	175 ± 13	170 ± 10		Day 14	28	27
Day 21	174 ± 21	151 ± 27	7	Day 21	28	26

Spleen weights were determined at time of sacrifice are represented as means \pm sd from four animals.

Table IV: Bone Marrow Myeloid: Erythoid Ratios

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Mouse #	Day 7	Day 14	Day 21
1	1.43	2.12	5.78
2	0.91	2.46	5.83
3	1.62	1.03	3.66
4		5.44	4.82
	1.32	2.76	5.02
	0.37	0.37	1.89
1	5.59	2.01	2.02
2	6.51	1.25	2.13
3	5.49	1.58	1.81
4	0.50	2.51	2.92
	4.52	1.86	2.22
	1.29	2.72	0.56
	1 2 3 4 2 3 3 3 4 3 3 4 3 4 3 4 4 4 4 4 4	1 1.43 2 0.91 3 1.62 4 1.32 0.37 1 5.59 2 6.51 3 5.49 4 0.50 4.52	1 1.43 2.12 2 0.91 2.46 3 1.62 1.03 4 5.44 1.32 2.76 0.37 0.37 1 5.59 2.01 2 6.51 1.25 3 5.49 1.58 4 0.50 2.51 4.52 1.86

All entries represent the number of myeloid cells per 1 erythroid cell. Normal mouse ratios are approximately 1:1 to 2:1.

Example 5

Additional Experiments Relating to

Hematopoietic Activity of Human CTLA-8

B18 (human CTLA-8) was tested for the ability to induce production of factors having hematopoietic activity in a factor-dependent cell proliferation assay using the

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human erythroleukemic cell line, TF-1 (Kitamura et al., J. Cell Physiol. 140:323 (1989)). The cells were initially grown in the presence of rhGMCSF (100 U/ml). The cells were fed three days prior to setting up the assay. The assay conditions were as follows:

5	cells/well	5000/200µ1
	incubation time	3 days
	pulse time	4 hours
	amount of tritiated thymidine	0.5μCi/well
	counting time	1 minute
10	replicates	2

B18 alone, conditioned medium (CM) from B18 induced HS-5 cells were assayed. Buffer alone, CM from HS-5 cells induced with buffer and CM from uninduced HS-5 cells were assayed as controls. Results are shown in Fig. 5. B18 (human CTLA-8) demonstrated an abilit to induce production of factors which induced TF-1 proliferation. This activity was substantially eliminated by the addition of anti-GMCSF antibodies. These data demonstrate that human CTLA-8 (B18) is able to induce hematopoiesis. Particularly, without being bound by any theory, it appears that human CTLA-8 (B18) induces production of GM-CSF and/or IL-3.

20 Example 6

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Ability of Human CTLA-8 to Induce Production of IL-6 and IL-8

MRC5 cells were incubated in the presence of human CTLA-8 (B18) and production of IL-6 and IL-8 were measured. Herpes CTLA-8 (IL-17) was used as a positive control. Applicants' human CTLA-8 (B18) demonstrated titratable production of both IL-6 and IL-8 (see Figs. 6 and 7).

All patent and literature references cited herein are incorporated by reference as if fully set forth.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Jacobs, Kenneth Kelleher, Kerry Carlin, McKeough Goldman, Samuel Pittman, Debra Mi, Sha Neben, Steven

Giannotti, JoAnn Golden'Fleet, Margaret

- (ii) TITLE OF INVENTION: Human CTLA-8 and Uses of CTLA-8-Related Proteins
 - (iii) NUMBER OF SEQUENCES: 9
 - (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Genetics Institute, Inc.
 - (B) STREET: 87 CambridgePark Drive
 - (C) CITY: Cambridge
 - (D) STATE: Massachusetts
 - (E) COUNTRY: USA
 - (F) ZIP: 02140
 - (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk

 - (B) COMPUTER: IBM PC compatible (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
 - (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
 - (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Brown, Scott A.
 - (B) REGISTRATION NUMBER: 32,724
 - (C) REFERENCE/DOCKET NUMBER: GI5262
 - (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (617) 498-8224
 - (B) TELEFAX: (617) 876-5851
- (2) INFORMATION FOR SEQ ID NO:1:
 - (i) SEOUENCE CHARACTERISTICS:
 - (A) LENGTH: 813 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: cDNA
 - (iii) HYPOTHETICAL: NO
 - (ix) FEATURE:

 - (A) NAME/KEY: CDS
 (B) LOCATION: 56..544

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:	
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CG ATA TTG GGG CTT GCC TTT CTG AGT GAG GCG GCA GCT CGG AAA ATC er Ile Leu Gly Leu Ala Phe Leu Ser Glu Ala Ala Ala Arg Lys Ile 20 25 30	154
CC AAA GTA GGA CAT ACT TTT TTC CAA AAG CCT GAG AGT TGC CCG CCT ro Lys Val Gly His Thr Phe Phe Gln Lys Pro Glu Ser Cys Pro Pro 35 40 45	202
TG CCA GGA GGT AGT ATG AAG CTT GAC ATT GGC ATC ATC AAT GAA AAC al Pro Gly Gly Ser Met Lys Leu Asp Ile Gly Ile Ile Asn Glu Asn 50 60 65	250
AG CGC GTT TCC ATG TCA CGT AAC ATC GAG AGC CGC TCC ACC TCC CCC In Arg Val Ser Met Ser Arg Asn Ile Glu Ser Arg Ser Thr Ser Pro 70 75 80	298
GG AAT TAC ACT GTC ACT TGG GAC CCC AAC CGG TAC CCC TCG GAA GTT rp Asn Tyr Thr Val Thr Trp Asp Pro Asn Arg Tyr Pro Ser Glu Val 85 90 95	346
TA CAG GCC CAG TGT AGG AAC TTG GGC TGC ATC AAT GCT CAA GGA AAG al Gln Ala Gln Cys Arg Asn Leu Gly Cys Ile Asn Ala Gln Gly Lys 100 105 110	394
AA GAC ATC TCC ATG AAT TCC GTT CCC ATC CAG CAA GAG ACC CTG GTC lu Asp Ile Ser Met Asn Ser Val Pro Ile Gln Gln Glu Thr Leu Val 115 120 125	442
TC CGG AGG AAG CAC CAA GGC TGC TCT GTT TCT TTC CAG TTG GAG AAG al Arg Arg Lys His Gln Gly Cys Ser Val Ser Phe Gln Leu Glu Lys 135 140 145	490
TG CTG GTG ACT GTT GGC TGC ACC TGC GTC ACC CCT GTC ATC CAC CAT al Leu Val Thr Val Gly Cys Thr Cys Val Thr Pro Val Ile His His 150 155 160	538
TG CAG TAAGAGGTGC ATATCCACTC AGCTGAAGAA GCTGTAGAAA TGCCACTCCT sal Gln	594
ACCCAGTGC TCTGCAACAA GTCCTGTCTG ACCCCCAATT CCCTCCACTT CACAGGACTC	65 ² 4
	714
	774
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(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 163 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

	()	ci) S	SEQUI	ENCE	DES	CRIPT	ON	SEC) ID	NO:2	2:	•				
Met 1	Thr	Val	Lys	Thr 5	Leu	His	Gly	Pro	Ala 10	Met	Val	Lys	Tyr	Leu 15	Leu	
Leu	Ser	Ile	Leu 20	Gly	Leu	Ala	Phe	Leu 25	Ser	Glu	Ala	Ala	Ala 30	Arg	Lys	
Ile	Pro	Lys 35	Val	Gly	His	Thr	Phe 40	Phe	Gln	Lys	Pro	Glu 45	Ser	Суѕ	Pro	
Pro	Val 50	Pro	Gly	Gly	Ser	Met 55	Lys	Leu	Asp	Ile	Gly 60	Ile	Ile	Asn	Glu	
Asn 65	Gln	Arg	Val	Ser	Met 70	Ser	Arg	Asn	Ile	Glu 75	Ser	Arg	Ser	Thr	Ser 80	
Pro	Trp	Asn	Tyr	Thr 85	Val	Thr	Trp	Asp	Pro 90	Asn	Arg	Tyr	Pro	Ser 95	Glu	٠
Val	Va1	Gln	Ala 100	Gln	Cys	Arg	Asn	Leu 105	Gly	Cys	Ile	Asn	Ala 110	Gln	Gly	
Lys	Glu	Asp 115	Ile	Ser	Met	Asn	Ser 120	Val	Pro	Ile	Gln	Gln 125	Glu	Thr	Leu	•
Val	Val 130	Arg	Arg	Lys	His	Gln 135	Gly	Cys	Ser	Val	Ser 140	Phe	Gln	Leu	Glu	
Lys 145	Val	Leu	Val	Thr	Val 150	Gly	Cys	Thr	Cys	Val 155	Thr	Pro	Val	Ile	His 160	
His	Val	Gln														
(2)	INF	ORMA'	rion	FOR	SEQ	ID 1	10:3	:								
	(i)	(,	A) L	ENGT	H: 4	CTER 51 ba	ase p	pair	5							
		(c) s'	rani	DEDN	ESS: line	doul									
	(ii)) MO	LECU	LE T	YPE:	cDN	A									
	(iii) HY:	РОТН	ETIC	AL: 1	10										
	(ix) FE	ATUR:	Ē:										•		
		(1	A) N B) L	AME/I	KEY:	CDS 6	155		,							
	(xi) SE	QUEN	CE D	escr:	IPTI(ON: S	SEQ :	ID N	0:3:						
CCA	CC A'	rg Toet Co	GC C' ys L	TG A' eu M	rg C' et L	rg T eu Le 5	rg c' eu L	rg C' eu L	ra C' eu L	eu A	AC C' sn L	rg G eu G	AG GG	CT AG	CA	47
GTG Val 15	AAG Lys	GCA Ala	GCG Ala	GTA Val	CTC Leu 20	ATC Ile	CCT Pro	CAA Gln	AGT Ser	TCA Ser 25	GTG Val	TGT Cys	CCA Pro	AAC Asn	GCC Ala 30	95
GAG Glu	GCC Ala	AAT Asn	AAC Asn	TTT Phe 35	CTC Leu	CAG Gln	AAC Asn	GTG Val	AAG Lys 40	GTC Val	AAC Asn	CTG Leu	AAA Lys	GTC Val 45	ATC Ile	143

			GCG Ala						191
			TGG Trp						239
			TGG Trp 85						287
			TTG Leu						335
			CTG Leu						383
			ATG Met						431
			GCG Ala	TAAT	Γ AA				461

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 150 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Cys Leu Met Leu Leu Leu Leu Leu Asn Leu Glu Ala Thr Val Lys
1 10 15

Ala Ala Val Leu Ile Pro Gln Ser Ser Val Cys Pro Asn Ala Glu Ala $20 \hspace{1cm} 25 \hspace{1cm} 30$

Asn Asn Phe Leu Gln Asn Val Lys Val Asn Leu Lys Val Ile Asn Ser 35 40 45

Leu Ser Ser Lys Ala Ser Ser Arg Arg Pro Ser Asp Tyr Leu Asn Arg 50 55 60

Ser Thr Ser Pro Trp Thr Leu Ser Arg Asn Glu Asp Pro Asp Arg Tyr 65 70 75 80

Pro Ser Val Ile Trp Glu Ala Gln Cys Arg His Gln Arg Cys Val Asn 85 90 95

Ala Glu Gly Lys Leu Asp His His Met Asn Ser Val Leu Ile Gln Gln 100 105 110

Glu Ile Leu Val Leu Lys Arg Glu Pro Glu Lys Cys Pro Phe Thr Phe 115 120 125

Arg Val Glu Lys Met Leu Val Gly Val Gly Cys Thr Cys Val Ser Ser 130 135 140 Ile Val Arg His Ala Ser 145

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 459 base pairs

(B) TYPE: nucleic acid (C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: CDS (B) LOCATION: 1..453

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

													CTG Leu			48
GAT Asp	TGT Cys	ATA Ile	GTA Val 20	AAG Lys	TCA Ser	GAA Glu	ATA Ile	ACT Thr 25	AGT Ser	GCA Ala	CAA Gln	ACC Thr	CCA Pro 30	AGA Arg	TGC Cys	96
TTA Leu	GCT Ala	GCT Ala 35	AAC Asn	AAT Asn	AGC Ser	TTT Phe	CCA Pro 40	CGG Arg	TCT Ser	GTG Val	ATG Met	GTT Val 45	ACT Thr	TTG Leu	AGC Ser	144
ATC Ile	CGT Arg 50	AAC Asn	TGG Trp	AAT Asn	ACC Thr	AGT Ser 55	TCT Ser	AAA Lys	AGG Arg	GCT Ala	TCA Ser 60	GAC Asp	TAC Tyr	TAC Tyr	AAT Asn	192
AGA Arg 65	TCT Ser	ACG Thr	TCT Ser	CCT Pro	TGG Trp 70	ACT Thr	CTC Leu	CAT His	CGC Arg	AAT Asn 75	GAA Glu	GAT Asp	CAA Gln	GAT Asp	AGA Arg 80	240
													GGA Gly			288
AAT Asn	GCT Ala	GAT Asp	GGG Gly 100	AAT Asn	GTA Val	GAC Asp	TAC Tyr	CAC His 105	ATG Met	AAC Asn	TCA Ser	GTC Val	CCT Pro 110	ATC Ile	CAA Gln	336
CAA Gln	GAG Glu	ATT Ile 115	CTA Leu	GTG Val	GTG Val	CGC Arg	AAA Lys 120	GGG Gly	CAT	CAA Gln	CCC Pro	TGC Cys 125	CCT Pro	AAT Asn	TCA Ser	384
TTT Phe	AGG Arg 130	CTA Leu	GAG Glu	AAG Lys	ATG Met	CTA Leu 135	GTG Val	ACT Thr	GTA Val	GGC Gly	TGC Cys 140	ACA Thr	TGC Cys	GTT Val	ACT Thr	432
					GTA Val 150		TAA	AAG								459

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

PCT/US96/11889

WO 97/04097

- (A) LENGTH: 151 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Thr Phe Arg Met Thr Ser Leu Val Leu Leu Leu Leu Ser Ile

Asp Cys Ile Val Lys Ser Glu Ile Thr Ser Ala Gln Thr Pro Arg Cys

Leu Ala Ala Asn Asn Ser Phe Pro Arg Ser Val Met Val Thr Leu Ser

Ile Arg Asn Trp Asn Thr Ser Ser Lys Arg Ala Ser Asp Tyr Tyr Asn

Arg Ser Thr Ser Pro Trp Thr Leu His Arg Asn Glu Asp Gln Asp Arg

Tyr Pro Ser Val Ile Trp Glu Ala Lys Cys Arg Tyr Leu Gly Cys Val

Asn Ala Asp Gly Asn Val Asp Tyr His Met Asn Ser Val Pro Ile Gln

Gln Glu Ile Leu Val Val Arg Lys Gly His Gln Pro Cys Pro Asn Ser 115 120 125

Phe Arg Leu Glu Lys Met Leu Val Thr Val Gly Cys Thr Cys Val Thr 130 140

Pro Ile Val His Asn Val Asp 145

- (2) INFORMATION FOR SEQ ID NO:7:
 - (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 28 base pairs

 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: cDNA
 - (iii) HYPOTHETICAL: NO
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CACAGGCATA CACAGGAAGA TACATTCA

- (2) INFORMATION FOR SEQ ID NO:8:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs

 - (B) TYPE: nucleic acid (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: cDNA

(xi) S	EQUENCE	DESCRIPTION	N: SEQ	ID	NO:8:	
TCTTGCTGGA	TGGGAAC	GGA ATTCA				

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 28 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9: ATACATTCAC AGAAGAGCTT CCTGCACA

28

- 5 What is claimed is:
 - 1. An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:
 - (a) the nucleotide sequence of SEQ ID NO:1 from nucleotide 146 to nucleotide 544;
- 10 (b) a nucleotide sequence capable of hybridizing to a nucleic acid sequence specified in (a);
 - (c) a nucleotide sequence varying from the sequence of the nucleotide sequence specified in (a) as a result of degeneracy of the genetic code; and
 - (d) an allelic variant of the nucleotide sequence specified in (a).
 - 2. The polynucleotide of claim 1 wherein said nucleotide sequence encodes a protein having CTLA-8 activity.
- 3. The polynucleotide of claim 1 wherein said nucleotide sequence is operably linked to an expression control sequence.
 - 4. The polynucleotide of claim 1 comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 55 to nucleotide 544.
- 5. A host cell transformed with the polynucleotide of claim 3.
 - 6. The host cell of claim 5, wherein said cell is a mammalian cell.
 - 7. A process for producing a human CTLA-8 protein, said process comprising:
 - (a) growing a culture of the host cell of claim 5 in a suitable culture medium; and
 - (b) purifying the human CTLA-8 protein from the culture.

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- 5 8. An isolated human CTLA-8 protein comprising an amino acid sequence selected from the group consisting of:
 - (a) the amino acid sequence of SEQ ID NO:2;
 - (b) the amino acid sequence of SEQ ID NO:2 from amino acids 11 to 163;
 - (c) the amino acid sequence of SEQ ID NO:2 from amino acids 29 to 163;
 - (d) the amino acid sequence of SEQ ID NO:2 from amino acids 31 to 163; and
 - (e) fragments of (a), (b), (c) or (d) having CTLA-8 activity.
 - 9. The protein of claim 8 comprising the amino acid sequence of SEQ ID NO:2.
- 10. The protein of claim 8 comprising the sequence from amino acid 29 to 163 of SEQID NO:2.
 - 11. A pharmaceutical composition comprising a human CTLA-8 protein of claim 8 and a pharmaceutically acceptable carrier.
 - 12. A human CTLA-8 protein produced according to the process of claim 7.
 - 13. A composition comprising an antibody which specifically reacts with a human CTLA-8 protein of claim 8.
 - 14. A method of treating a mammalian subject comprising administering a therapeutically effective amount of a composition of claim 11.
 - 15. A method of treating a mammalian subject comprising administering a therapeutically effective amount of a composition comprising a pharmaceutically acceptable carrier and a protein comprising an amino acid sequence selected from the group consisting of:
 - (a) the amino acid sequence of SEQ ID NO:4;

10

- (b) the amino acid sequence of SEQ ID NO:4 from amino acids 18 to 150; and
 - (c) fragments of (a) or (b) having CTLA-8 activity.
 - 16. The method of claim 15 wherein said protein comprises the amino acid sequence of SEQ ID NO:4.
- 17. The method of claim 15 wherein said protein comprises the amino acid sequence of SEO ID NO:4 from amino acids 18 to 150.
- 18. A method of treating a mammalian subject administering a therapeutically effective amount of a composition comprising a pharmaceutically acceptable carrier and a protein comprising an amino acid sequence selected from the group consisting of:
 - (a) the amino acid sequence of SEQ ID NO:6;
 - (b) the amino acid sequence of SEQ ID NO:6 from amino acids 19 to 151; and
 - (c) fragments of (a) or (b) having CTLA-8 activity.
 - 19. The method of claim 18 wherein said protein comprises the amino acid sequence of SEQ ID NO:6.
- 20. The method of claim 18 wherein said protein comprises the amino acid sequence of SEQ ID NO:6 from amino acids 19 to 151.
 - 21. The polynucleotide of claim 1 comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 86 to nucleotide 544.
- The protein of claim 8 comprising the sequence from amino acid 11 to 163 of SEQ ID NO:2.

- 5 23. A method of treating a mammalian subject comprising administering a therapeutically effective amount of a composition comprising a pharmaceutically acceptable carrier and IL-17 or an active fragment thereo.
- 24. The method of claim 14, 15, 18 or 23 wherein said subject is treated to produce an effect selected from the group consisting of inhibition of angiogenesis, inhibition of growth or proliferation of vascular endothelial cells, inhibition of tumor growth, inhibition of angiogenesis-dependent tissue growth, proliferation of myeloid cells or progenitors, proliferation of erythroid cells or progenitors, proliferation of lymphoid cells or progenitors, induction of IFNγ production, induction of IL-3 production and induction of GM-CSF production.
 - 25. The composition of claim 3 wherein said polynucleotide is contained in a vector suitable for *in vivo* expression in a mammalian subject.
- 26. The polynucleotide of claim 1 comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 139 to nucleotide 544.
 - 27. The protein of claim 8 comprising the sequence from amino acid 31 to 163 of SEQ ID NO:2.

\$1 \$3		133
2IVKS EITS AQ-TPRCL-AANNSFPRSVMVTLSIRNWNTSSKRASDYYNRSTSP	2 WTLHRNEDQDRYPSVIWEAKCRYLGCVNADGNVDYHMNSVPIQQEILVVRKGHQPCPNSF	RUEKHLVTVGCTCVTPIVHNVD
8AVLIPQ-SSVCPNAEANNELONVKVNLKVINSLSKASSRRPSDYLNRSTSP	8 WTLSRNEDPDRYPSVIWEAQCRHQRCVNAEGKLDHHMNSVLLQQEILVLKREPEKCPFTF	RRVEKHLVGVGCTCVSSIVRHAS
1 AARKIPKVGHTFFQKPESCPPVPGGSMKLDIGIINENQRVSMSRNIESRSTSP	11 WNYTVTWDPNRYPSEVVQAQCRNLGCINAQGKEDISHNSVPIQQETLVVRRKHQGCSVSF	NQLEKVLVTVGCTCVTPVIHHVQ
Ksvie_2	Hsvie_2	Hsvie_2
Musctla8	Musctla8	Musctla8
B18_F1	B18_F1	B18_F1

FIGURE

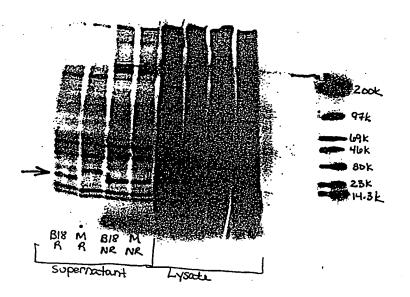
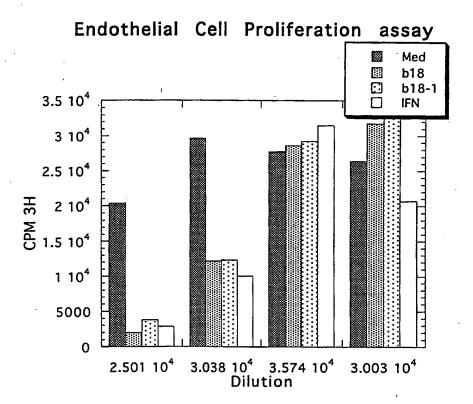


FIGURE 2



Fig, 3

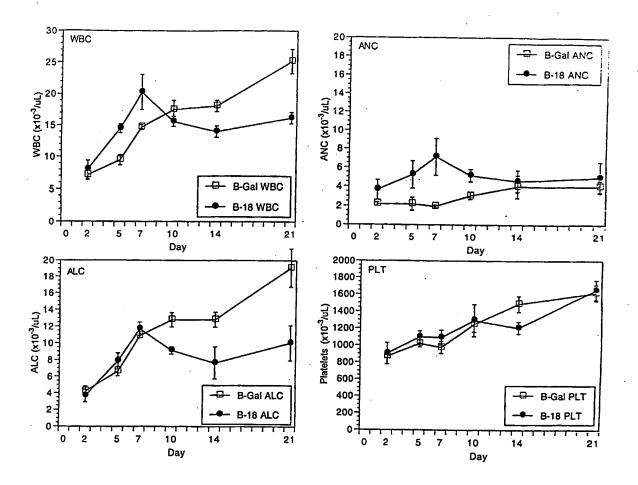
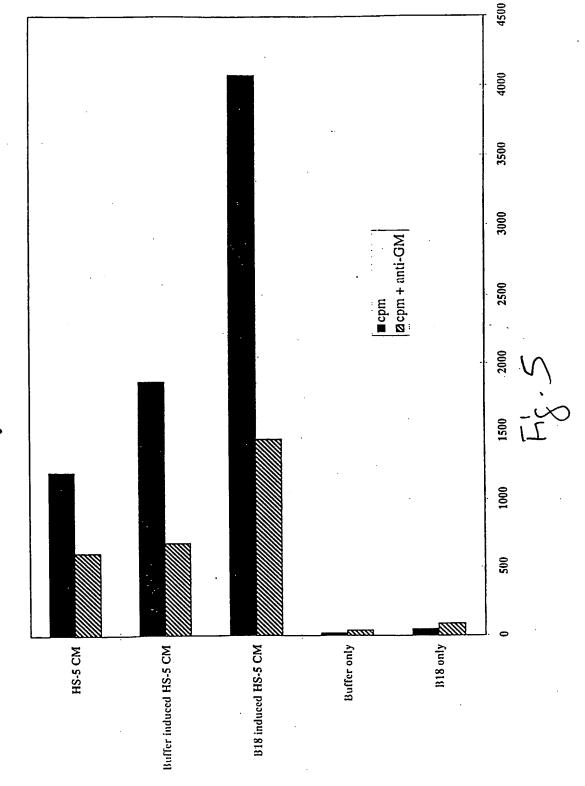
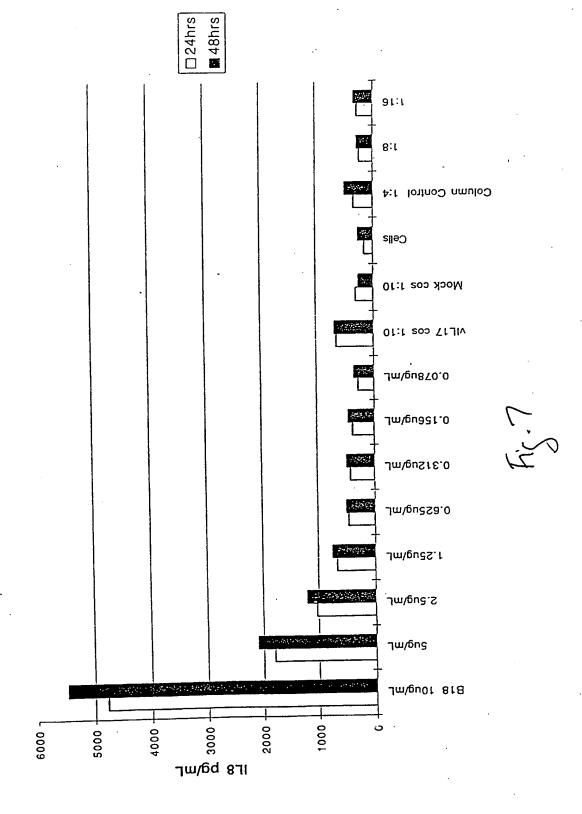


Fig. 24

TF-1 activity in CIM from B18 induced HS-5 cells



IL8 Production from B18 on MRC5 Cells - 24 & 48hrs





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(51) International Patent Classification 6: C12N 15/19, 15/12, 15/24, C07K 14/52, 14/725, 14/54, A61K 38/17, 38/19, 38/20, C12N 5/10, C07K 16/24, 16/28, A61K 48/00

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PCT/US96/11889

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18 July 1996 (18.07.96)

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NL, PT, SE).

(30) Priority Data:

08/504,032 08/514.014 19 July 1995 (19.07.95) 11 August 1995 (11.08.95) US US

(71) Applicant: GENETICS INSTITUTE, INC. [US/US]; 87 Cambridge Park Drive, Cambridge, MA 02140 (US).

(72) Inventors: JACOBS, Kenneth; 151 Beaumont Avenue, Newton, MA 02160 (US). KELLEHER, Kerry; 50 Hurley Circle, Marlborough, MA 01752 (US). CARLIN, McKeough; 16 Chauncy Street #2, Cambridge, MA 02138 (US). GOLD-MAN, Samuel; 9 Mohawk Drive, Acton, MA 01720 (US). PITTMAN, Debra; 20 N. Shore Road, Windham, NH 03087 (US). MI, Sha; 4 Vernon Road, Belmont, MA 02178 (US). NEBEN, Steven; 13 Duggan Road, Acton, MA 01720 (US). GIANNOTTI, JoAnn; 409 Arlington Street, Acton, MA 01720 (US). GOLDEN-FLEET, Margaret; 19 Usher Road, Medford, MA 02155 (US).

(74) Agent: BROWN, Scott, A.; Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140 (US).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

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(54) Title: HUMAN CTLA-8 AND USES OF CTLA-8-RELATED PROTEINS

(57) Abstract

Polynucleotides encoding human CTLA-8 and related proteins are disclosed. Human CTLA-8 proteins and methods for their production are also disclosed. Methods of treatment using human CTLA-8 proteins, rat CTLA-8 proteins and herpesvirus herpes CTLA-8 proteins are also provided.

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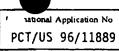
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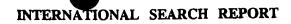
s sauchal Application No PCT/US 96/11889

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IPC 6	FICATION OF SUBJECT MATTER C12N15/19 C12N15/12 C12N15 C07K14/54 A61K38/17 A61K38 C07K16/24 C07K16/28 A61K48 International Patent Classification (IPC) or to both national cl	3/19 A61K38 3/00	/52 C07K1 /20 C12N5	
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Minimum do IPC 6	ocumentation searched (classification system followed by classification sy			
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Electronic d	ata base consulted during the international search (name of data	s base and, where practic	ll, search terms used)	
C. DOCUM	IENTS CONSIDERED TO BE RELEVANT			
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х	WO 95 18826 A (SCHERING CORP ; SANTE RECH MED (FR)) 13 July 1 cited in the application	INST NAT 995		1-3,5-7, 12, 15-20, 23-25
A	see page 3, line 21 - page 4, see page 12, line 1 - page 16, see page 30, line 1-13 Seq.ID:2 see page 53 Seq.ID:4 see page 55 Seq.ID:5 see page 56 Seq.ID:7/8 see page 58 - page 59	line 11 line 6		11,13,14
		-/		
<u> </u>	ther documents are listed in the continuation of box C.	X Patent fam	ily members are listed	in annex.
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Category Citat	ion of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
, augus)	on of sociations, with interesting where appropriate, or the relevant passages	
	THE JOURNAL OF IMMUNOLOGY, 101. 150, no. 12, 15 June 1993, 10ages 5445-5456, XP002035505	15-20
ļ F	OUVIER E. ET AL.: "CTLA-8, cloned from in activated T cell, bearing AU-rich	
, n	messenger RNA instability sequences, and nomologous to a Herpesvirus Saimiri gene"	
	ited in the application see page 5445 - page 5446, line 11	.
-	see page 5449, right-hand column, line 26 page 5450	
2	see page 5451; figure 3 see page 5453, left-hand column, line 10 - right-hand column, line 16	
	OURNAL OF VIROLOGY, ol. 66, no. 8, August 1992,	1
F	ages 5047-5058, XPO00615399 LBRECHT J -C ET AL: "PRIMARY STRUCTURE	
A S	F THE HERPESVIRUS SAIMIRI GENOME" eq.ID:1 from nt.552 to nt.217 (reverse	18-20
· >	rientation) is 61.4% homologous to 64346 from nt.26931 to nt.27266. see page 5048, right-hand column, line	
1 0	5-18 RF13	
	ee page 5049; table 1 O 97 07198 A (GENETICS INST) 27 February	1-14,21,
1	997 ee page 8, line 15-31	22,24-27
5	ee page 9, line 26 - page 11, line 7 ee page 13, line 16-27 ee page 18, line 10 - page 19, line 25	
9	ee page 25, line 11-15 ee page 27, line 5-17	
9	ee page 31, line 27-34 eq.ID:11-12 ee page 49 - page 51	
P,X J	OURNAL OF IMMUNOLOGY,	1-3,5-7,
l V	ol. 155, no. 12, 15 December 1995, ages 5483-5486, XP000602481 AO Z ET AL: "HUMAN IL-17: A NOVEL	23
5	YTOKINE DERIVED FROM T CELLS" ee page 5483	
S	ee page 5484; figure 1	
	-/	



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		PC1/US 96/11009
	ction) DOCUMENTS CONSIDERED TO BE RELEVANT Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
legory *	Citation of document, with indication, where appropriate, or die relevant passages	•
, X	THE JOURNAL OF EXPERIMENTAL MEDICINE, vol. 183, no. 6, 1 June 1996, pages 2593-2603, XP002035506 FOSSIEZ F. ET AL.: "T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines" see abstract	1-3,5-7, 15-20, 23,24
	see page 2594; figure 1	·
Ρ,Χ	IMMUNITY, vol. 3, no. 6, 1 December 1995, pages 811-821, XP000579309 YAO Z ET AL: "HERPESVIRUS SAIMIRI ENCODES A NEW CYTOKINE, IL-17, WHICH BINDS TO A NOVEL CYTOKINE RECEPTOR" see page 811 see page 815, left-hand column, line 50 - page 818, left-hand column	15-20, 23,24
E	WO 96 29408 A (IMMUNEX CORP) 26 September 1996 see page 1, line 1 - page 2, line 33 Seq.ID:8 see page 36	18-20
P,X	GENE, vol. 168, no. 2, 12 February 1996, pages 223-225, XP002035631 YAO ET AL.: "Complete nucleotide sequence of the mouse CTLA8 gene" see page 225; figure 2	1,2
·		
		·

International application No.

PCT/US 96/11889

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)
This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
Claims Nos.: 14-20,23,24 because they relate to subject matter not required to be searched by this Authority, namely: Remark: Although claims 14-20,2,24 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the
alleged effects of the compound/composition. 2. Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows:
see continuation-sheet
1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. X As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Remark on Protest The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.

International Application No. PCT/US 96/ 11889

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

- 1) claims 1-14, 21, 22, 25-27 all totally; claim 24 partially.

 Isolated polynucleotide comprising Seq.ID:1, homologue sequences and derivates. Vectors and transformed host cells. Process for producing human recombinant CTLA-8 protein. Isolated human CTLA-8 protein as in Seq.ID:2 and fragments. Pharmaceutical compositions and uses in therapy. Antibodies.
 - 2) claims 15-17 all totally; claim 24 partially.

 Therapeutical uses of the protein as in Seq.ID:4 or fragments.
 - claims 18-20 all totally; claim 24 partially.
 Therapeutical uses of the protein as in Seq.ID:6 or fragments.
 - 4) claims 23 all totally; claim 24 partially.

 Therapeutical uses of IL-17 or active fragments.

I national Application No PCT/US 96/11889

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9518826 A	13-07-95	AU 1520895 A EP 0733069 A JP 9501572 T	01-08-95 25-09-96 18-02-97
WO 9707198 A	27-02-97	AU 6712396 A AU 6768596 A WO 9704097 A	18-02-97 12-03-97 06-02-97
WO 9629408 A	26-09-96	AU 5526396 A	08-10-96





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(51) International Patent Classification ⁶:
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(30) Priority Data:

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11 August 1995 (11.08.95) US

US

(71) Applicant: GENETICS INSTITUTE, INC. [US/US]; 87 Cambridge Park Drive, Cambridge, MA 02140 (US).

(72) Inventors: JACOBS, Kenneth; 151 Beaumont Avenue, Newton, MA 02160 (US). KELLEHER, Kerry; 50 Hurley Circle, Marlborough, MA 01752 (US). CARLIN, McKeough; 16 Chauncy Street #2, Cambridge, MA 02138 (US). GOLDMAN, Samuel; 9 Mohawk Drive, Acton, MA 01720 (US). PITTMAN, Debra; 20 N. Shore Road, Windham, NH 03087 (US). MI, Sha; 4 Vernon Road, Belmont, MA 02178 (US). NEBEN, Steven; 13 Duggan Road, Acton, MA 01720 (US). GIANNOTTI, JoAnn; 409 Arlington Street, Acton, MA 01720 (US). GOLDEN-FLEET, Margaret; 19 Usher Road, Medford, MA 02155 (US).

(74) Agent: BROWN, Scott, A.; Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140 (US). (81) Designated States: AU, CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report: 12 September 1997 (12.09.97)

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	Estonia	MD	Republic of Moldova	UA	Ukraine
EE		MG	Madagascar	UG	Uganda
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France	MR	Mauritania	VN	Viet Nam
GA	Gabon	IVIA	***************************************		

HUMAN CTLA-8 AND USES OF CTLA-8-RELATED PROTEINS

This application is a continuation-in-part of application Ser. No. 08/504,032, filed July 19, 1995, and a continuation-in-part of application Ser. No. 08/514,014, filed August 11, 1995.

Field of the Invention

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The present invention relates to human CTLA-8 proteins, nucleic acids encoding such proteins, methods of treatment using such proteins. The invention also relates to the use of rat CTLA-8 proteins and herpesvirus *Saimiri* ORF13 proteins in methods of treatment.

Background of the Invention

Cytokines are secreted proteins which act on specific hematopoietic target cells to cause a differentiation event or on other target cells to induce a particular physiological response, such as secretion of proteins characteristic of inflammation. Cytokines, also variously known as lymphokines, hematopoietins, interleukins, colony stimulating factors, and the like, can be important therapeutic agents, especially for diseases or conditions in which a specific cell population is depleted. For example, erythropoietin, G-CSF, and GM-CSF, have all become important for treatment of anemia and leukopenia, respectively. Other cytokines such as interleukin-3, interleukin-6, interleukin-11 and interleukin-12 show promise in treatment of conditions such as thrombocytopenia and modulation of immune response.

For these reasons a significant research effort has been expended in searching for novel cytokines and cloning the DNAs which encode them. In the past, novel cytokines were identified by assaying a particular cell such as a bone marrow cell, for a measurable response, such as proliferation. The search for novel cytokines has thus been limited by the assays available, and if a novel cytokine has an activity which is unmeasurable by a known assay, the cytokine remains undetectable. In a newer approach, cDNAs encoding cytokines have been detected using the polymerase chain

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reaction (PCR) and oligonucleotide primers having homology to shared motifs of known cytokines or their receptors. The PCR approach is also limited by the necessity for knowledge of previously cloned cytokines in the same protein family. Cytokines have also been cloned using subtractive hybridization to construct and screen cDNA libraries, or they can potentially be cloned using PCR followed by gel electrophoresis to detect differentially expressed genes. The subtractive hybridization methods are based on the assumption that cytokine mRNAs are those that are differentially expressed, and these methods do not require any prior knowledge of the sequence of interest. However, many cytokines may be encoded by mRNAs which are not differentially expressed, and thus are undetectable using these methods.

It would be desirable to develop new methods for identifying novel cytokines and other secreted factors and to isolate polynucleotides encoding them.

Summary of the Invention

In developing the present invention, methods were employed which selectively identify polynucleotides which encode secreted proteins. One such polynucleotide was isolated which encodes "human CTLA-8." In accordance with the present invention, polynucleotides encoding human CTLA-8 and active fragments thereof are disclosed. "CTLA-8" is used throughout the present specification to refer to both proteins and polynucleotides encoding those proteins and to refer to proteins and polynucleotides from all mammalian species.

In certain embodiments, the present invention provides an isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:

- (a) the nucleotide sequence of SEQ ID NO:1 from nucleotide 146 to nucleotide 544;
- (b) a nucleotide sequence capable of hybridizing to a nucleic acid sequence specified in (a);
- (c) a nucleotide sequence varying from the sequence of the nucleotide sequence specified in (a) as a result of degeneracy of the genetic code; and
 - (d) an allelic variant of the nucleotide sequence specified in (a).

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Preferably, the polynucleotide of the invention encodes a protein having CTLA-8 activity. In other embodiments the polynucleotide is operably linked to an expression control sequence. In other preferred embodiments, the polynucleotide is contained in a vector suitable for *in vivo* expression in a mammalian subject. Polynucleotides comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 55 to nucleotide 544, the nucleotide sequence of SEQ ID NO:1 from nucleotide 139 to nucleotide 544 or the nucleotide sequence of SEQ ID NO:1 from nucleotide 86 to nucleotide 544 are particularly preferred.

Host cells transformed with the polynucleotides of the invention are also provided, including mammalian cells.

Processes are also provided for producing a human CTLA-8 protein, said processes comprising:

- (a) growing a culture of the host cell of the invention in a suitable culture medium; and
 - (b) purifying the human CTLA-8 protein from the culture.

Isolated human CTLA-8 protein is also provided which comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:2;
- (b) the amino acid sequence of SEQ ID NO:2 from amino acids 11 to 163;
- (c) the amino acid sequence of SEQ ID NO:2 from amino acids 29 to 163;
- (d) the amino acid sequence of SEQ ID NO:2 from amino acids 31 to 163; and
 - (e) fragments of (a), (b), (c) or (d) having CTLA-8 activity.
- Proteins comprising the amino acid sequence of SEQ ID NO:2 and comprising the sequence from amino acids 29 to 163, from amino acid 31 to 163, or from amino acids 11 to 163 of SEQ ID NO:2 are particularly preferred. Preferably, the protein has CTLA-8 activity. Pharmaceuticals composition comprising a human CTLA-8 protein of the invention and a pharmaceutically acceptable carrier are also provided.
 - Compositions are also disclosed which comprise an antibody which specifically reacts with a human CTLA-8 protein of the invention.

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Methods of treating a mammalian subject are also provided which comprise administering a therapeutically effective amount of a pharmaceutical composition comprising a human CTLA-8 protein.

Rat CTLA-8 and active (i.e., having CTLA-8 activity) fragments thereof may also be used in such methods of treatment. Preferably the rat protein is administered as a composition comprising a pharmaceutically acceptable carrier and a protein comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:4;
- (b) the amino acid sequence of SEQ ID NO:4 from amino acids 18 to 150; and
- (c) fragments of (a) or (b) having CTLA-8 activity.

Herpesvirus Saimiri ORF13, referred to herein as "herpes CTLA-8", and active (i.e., having CTLA-8 activity) fragments thereof and active fragments thereof may also be used in such methods of treatment. Preferably the herpes CTLA-8 protein is administered as a composition comprising a pharmaceutically acceptable carrier and a protein comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:6;
- (b) the amino acid sequence of SEQ ID NO:6 from amino acids 19 to 151;and
- (c) fragments of (a) or (b) having CTLA-8 activity.

The invention also provides a method of treating a mammalian subject comprising administering a therapeutically effective amount of a composition comprising a pharmaceutically acceptable carrier and IL-17 or an active fragment thereof.

In methods of treatment provided by the present invention, preferably the subject is treated to produce an effect selected from the group consisting of inhibition of angiogenesis, inhibition of growth or proliferation of vascular endothelial cells, inhibition of tumor growth, inhibition of angiogenesis-dependent tissue growth, proliferation of myeloid cells or progenitors, proliferation of erythroid cells or progenitors, proliferation of IFN production, induction of IL-3 production and induction of GM-CSF production.

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Brief Description of the Figures

Fig. 1 is a comparison of homologous regions of the amino acid sequences of human CTLA-8 (indicated as "B18_F1"), rat CTLA-8 (indicated as "Musctla8") and herpes CTLA-8 (indicated as "Hsvie_2").

Fig. 2 depicts autoradiographs demonstrating expression of human CTLA-8 in COS cells.

Fig. 3 presents data relating to the ability of human CTLA-8 to inhibit angiogenesis.

Figs. 4 and 5 present data relating to the ability of human CTLA-8 to produce or induce hematopoietic activity.

Figs. 6 and 7 present data demonstrating the ability of human CTLA-8 to induce production of IL-6 and IL-8.

Detailed Description of Preferred Embodiments

The inventors of the present application have identified and provided a polynucleotide encoding a human CTLA-8 protein. SEQ ID NO:1 provides the nucleotide sequence of a cDNA encoding the human CTLA-8 protein. SEQ ID NO:2 provides the amino acid sequence of the human CTLA-8 protein. Alternatively, the initiating methionine may be at amino acid 11 of SEQ ID NO:2. On the basis of amino terminal sequencing, the mature protein sequence is believed to begin at amino acid 31 of SEQ ID NO:2 (encoded by the sequence beginning with nucleotide 146 of SEO ID NO:1).

The region from amino acid 29 to amino acid 163 of human CTLA-8 (SEQ ID NO:2) shows marked homology to portions of rat CTLA-8 (amino acids 18 to 150 of SEQ ID NO:4) and herpesvirus *Saimiri* ORF13 ("herpes CTLA-8") (amino acids 19 to 151 of SEQ ID NO:5). A cDNA sequence encoding rat CTLA-8 is listed at SEQ ID NO:3 and its corresponding amino acid sequence is reported at SEQ ID NO:4. A cDNA sequence encoding herpes CTLA-8 is listed at SEQ ID NO:5 and its corresponding amino acid sequence is reported at SEQ ID NO:6. Homology between rat CTLA-8 and herpes CTLA-8 was reported by Rouvier et al., J. Immunol. 1993, 150, 5445-5456.

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Applicants had previously incorrectly identified the rat sequences of SEQ ID NO:3 and SEQ ID NO:4 as applying to murine CTLA-8. Applicants' human CTLA-8 (B18) does also show homolgy to the true murine CTLA-8 sequence.

Golstein et al. (WO95/18826; Fossiez et al., Microbial Evasion and Subversion of Immunity 544:3222 (Abstract)) have also reported a species they initially identified as "human CTLA-8." However, examination of the sequence of the Golstein et al. species and the human CTLA-8 (B18) sequence of the present invention readily reveals that they are two different proteins, although they are homologous with each other and with the rat CTLA-8 and herpes CTLA-8 identified herein. The Golstein et al. species has now been renamed as interleukin-17 (IL-17). Because of the homology between applicants' human CTLA-8 (B18) and IL-17, these proteins are expected to share some activities.

It has also been preliminarily determined that human CTLA-8 (B18) forms homodimers when expressed. As a result, human CTLA-8 proteins may possess activity in either monomeric or dimeric forms. Human CTLA-8 proteins can also be produced as heterodimers with rat and herpes CTLA-8 proteins and with human IL-17. These heterodimers are also expected to have activities of the proteins of which they are comprised.

Forms of human CTLA-8 protein of less than full length are encompassed within the present invention and may be produced by expressing a corresponding fragment of the polynucleotide encoding the human CTLA-8 protein (SEQ ID NO:1). These corresponding polynucleotide fragments are also part of the present invention. Modified polynucleotides as described above may be made by standard molecular biology techniques, including site-directed mutagenesis methods which are known in the art or by the polymerase chain reaction using appropriate oligonucleotide primers.

For the purposes of the present invention, a protein has "CTLA-8 activity" if it either (1) displays biological activity in a factor-dependent cell proliferation assay (preferably an assay in which full-length the corresponding species full-length CTLA-8 is active) (including without limitation those assays described below), or (2) induces expression or secretion of γ -IFN, or (3) displays chemoattractant of chemotactic activity in a chemoattraction or chemotaxis assay (preferably as assay in which full-

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length the corresponding species full-length CTLA-8 is active) or (4) induces expression of secretion of IL-3 or GM-CSF.

Human CTLA-8 protein or fragments thereof having CTLA-8 activity may be fused to carrier molecules such as immunoglobulins. For example, human CTLA-8 protein may be fused through "linker" sequences to the Fc portion of an immunoglobulin.

The invention also encompasses allelic variations of the nucleotide sequence as set forth in SEQ ID NO:1, that is, naturally-occurring alternative forms of the isolated polynucleotide of SEQ ID NO:1 which also encode human CTLA-8 or CTLA-8 proteins having CTLA-8 activity. Also included in the invention are isolated polynucleotides which hybridize to the nucleotide sequence set forth in SEQ ID NO:1 under highly stringent (0.2xSSC at 65°C), stringent (e.g. 4xSSC at 65°C or 50% formamide and 4xSSC at 42°C), or relaxed (4xSSC at 50°C or 30-40% formamideand 4xSSC at 42°C) conditions. Isolated polynucleotides which encode human CTLA-8 protein but which differ from the nucleotide sequence set forth in SEQ ID NO:1 by virtue of the degeneracy of the genetic code are also encompassed by the present invention. Variations in the nucleotide sequence as set forth in SEQ ID NO:1 which are caused by point mutations or by induced modifications which enhance CTLA-8 activity, half-life or production level are also included in the invention.

The isolated polynucleotides of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman et al., Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the CTLA-8 protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, Methods in Enzymology 185, 537-566 (1990). As defined herein "operably linked" means enzymatically or chemically ligated to form a covalent bond between the isolated polynucleotide of the invention and the expression control sequence, in such a way that the CTLA-8 protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

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A number of types of cells may act as suitable host cells for expression of the human CTLA-8 protein. Any cell type capable of expressing functional human CTLA-8 protein may be used. Suitable mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK, Rat2, BaF3, 32D, FDCP-1, PC12 or C2C12 cells.

The human CTLA-8 protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, e.g., Invitrogen, San Diego, California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. Soluble forms of the human CTLA-8 protein may also be produced in insect cells using appropriate isolated polynucleotides as described above.

Alternatively, the human CTLA-8 protein may be produced in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Suitable yeast strains include Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces strains, Candida, or any yeast strain capable of expressing heterologous proteins. Suitable bacterial strains include Escherichia coli, Bacillus subtilis, Salmonella typhimurium, or any bacterial strain capable of expressing heterologous proteins.

The human CTLA-8 protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a polynucleotide sequence encoding the human CTLA-8 protein.

The human CTLA-8 protein of the invention may be prepared by growing a culture of transformed host cells under culture conditions necessary to express the desired protein. The resulting expressed protein may then be purified from the culture medium or cell extracts. Soluble forms of the human CTLA-8 protein of the invention

can be purified from conditioned media. Membrane-bound forms of human CTLA-8 protein of the invention can be purified by preparing a total membrane fraction from the expressing cell and extracting the membranes with a non-ionic detergent such as Triton X-100.

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The human CTLA-8 protein can be purified using methods known to those skilled in the art. For example, the human CTLA-8 protein of the invention can be concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration medium. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred (e.g., S-Sepharose® columns). The purification of the human CTLA-8 protein from culture supernatant may also include one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; or by hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or by immunoaffinity chromatography. Finally, one or more reversephase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the human CTLA-8 protein. Some or all of the foregoing purification steps, in various combinations or with other known methods, can also be employed to provide a substantially purified isolated recombinant protein.

Preferably, the human CTLA-8 protein is purified so that it is substantially free of other mammalian proteins.

It is believed that human CTLA-8, active fragments and variants thereof, and CTLA-8 related proteins (such as, for example, rat CTLA-8 and herpes CTLA-8) (collectively "CTLA-8 proteins") possess or induce cytokine activities. Human

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CTLA-8 expression correlated with γ-IFN expression in induced primary cells and can induce the expression of IL-3 and/or GM-CSF, which expression can in turn produce effects associated with the induced cytokine. Therefore, human CTLA-8 and CTLA-8 related proteins may have an effect on proliferation or function of myeloid cells, erythroid cells, lymphoid cells and their progenitors. Human CTLA-8 proteins may also play a role in formation of platelets or their progenitors.

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnolli et al., J. Immunol. 145:1706-1712, 1990; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli, et al., J. Immunol. 149:3778-3783, 1992; Bowman et al., J. Immunol. 152: 1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A.M. and Shevach, E.M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human Interferon γ, Schreiber, R.D.

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In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L.S. and Lipsky, P.E. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988; Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human interleukin 6 - Nordan, R. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11 - Bennett, F., Giannotti, J., Clark, S.C. and Turner, K. J. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9 - Ciarletta, A., Giannotti, J., Clark, S.C. and Turner, K.J. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober

Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors;

Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various

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immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpes viruses, mycobacteria, leshmania, malaria and various fungal infections such as candida. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally would be indicated, i.e., in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, asthma and related respiratory conditions), may also be treatable using a protein of the present invention.

A protein of the present invention may also suppress chronic or acute inflammation, such as, for example, that associated with infection (such as septic shock or systemic inflammatory response syndrome (SIRS)), inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-1 (such as the effect demonstrated by IL-11).

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte

Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowmanet al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

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Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: *In vitro* antibody production, Mond, J.J. and Brunswick, M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by denritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimenal Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine

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169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentarily to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility

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in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either *in-vivo* or *ex-vivo* (i.e. in conjunction with bone marrow transplantation) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embyronic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M.G. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I.K. and Briddell, R.A. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, NY. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R.E. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 1-21, Wiley-Liss, Inc.., New York, NY. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, NY. 1994; Long term culture initating cell assay, Sutherland, H.J. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, NY. 1994.

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CTLA-8 proteins are useful in the treatment of various immune deficiencies and disorders (including SCID), e.g., in regulating (up or down) growth, proliferation and/or activity of T and/or B lymphocytes, as well as the cytolytic activity of NK cells. These immune deficiencies may be caused by viral (e.g., HIV) as well as bacterial infections, or may result from autoimmune disorders. More specifically, infectious diseases caused by viral, bacterial, fungal or other infection may be treatable using CTLA-8 proteins, including infections by HIV, hepatitis, influenza, CMV, herpes, mycobacterium, leishmaniasis, malaria and various fungal infections (such as candida). Of course, in this regard, the CTLA-8 proteins may also be useful where a boost to the immune system generally would be indicated, i.e., in the treatment of cancer or as an adjuvant to vaccines. Autoimmune disorders which may be treated using factors of the present invention include, for example, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes melitis and autoimmune inflammatory eye disease. The CTLA-8 proteins are also expected to be useful in the treatment of allergic reactions and conditions.

CTLA-8 proteins are also expected to have chemotactic activity. A protein or peptide has "chemotactic activity," as used herein, if it can stimulate, directly or indirectly, the directed orientation or movement of cells, including myeloid and lymphoid cells. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells (particularly T-cells). Whether a particular protein or peptide has chemotactic activity for cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

CTLA-8 proteins also inhibit growth and proliferation of vascular endothelial cells. As a result, human CTLA-8 proteins are effective in inhibiting angiogenesis (i.e., vascular formation). This activity will also be useful in the treatment of tumors and other conditions in which angiogenesis in involved. Inhibition of angiogenesis by human CTLA-8 proteins will also result in inhibition or prevention of the condition to which normal angiogenesis would contribute.

Isolated CTLA-8 proteins, purified from cells or recombinantly produced, may be used as a pharmaceutical composition when combined with a pharmaceutically

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acceptable carrier. Such a composition may contain, in addition to CTLA-8 protein and carrier, diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-15, G-CSF, γ-IFN, stem cell factor, and erythropoietin. The pharmaceutical composition may contain thrombolytic or anti-thrombotic factors such as plasminogen activator and Factor VIII. The pharmaceutical composition may further contain other antiinflammatory agents. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with CTLA-8 protein, or to minimize side effects caused by the CTLA-8 protein. Conversely, CTLA-8 protein may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

The pharmaceutical composition of the invention may be in the form of a liposome in which CTLA-8 protein is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers which in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323, all of which are incorporated herein by reference.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, e.g., amelioration of symptoms of,

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healing of, or increase in rate of healing of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of CTLA-8 protein is administered to a mammal. CTLA-8 protein may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines, other hematopoietic factors or vaccine components (such as antigens or other adjuvants), CTLA-8 protein may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering CTLA-8 protein in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of CTLA-8 protein used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, or cutaneous, subcutaneous, or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of CTLA-8 protein is administered orally, CTLA-8 protein will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% CTLA-8 protein, and preferably from about 25 to 90% CTLA-8 protein. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol,

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propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of CTLA-8 protein, and preferably from about 1 to 50% CTLA-8 protein.

When a therapeutically effective amount of CTLA-8 protein is administered by intravenous, cutaneous or subcutaneous injection, CTLA-8 protein will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to CTLA-8 protein an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additive known to those of skill in the art.

The amount of CTLA-8 protein in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of CTLA-8 protein with which to treat each individual patient. Initially, the attending physician will administer low doses of CTLA-8 protein and observe the patient's response. Larger doses of CTLA-8 protein may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not generally increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.1 µg to about 100 mg of CTLA-8 protein per kg body weight, preferably about 0.1 µg to about 100 µg of CTLA-8 protein per kg body weight, most preferably preferably about 0.1 µg to about 100 µg of CTLA-8 protein per kg body weight, most preferably preferably about 0.1 µg to about 100 µg of CTLA-8 protein per kg body weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It

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is contemplated that the duration of each application of the CTLA-8 protein will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

CTLA-8 protein of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the CTLA-8 protein and which may inhibit CTLA-8 binding to its receptor. Such antibodies are also useful for performing diagnostics assays for CTLA-8 in accordance with known methods. Such antibodies may be obtained using the entire CTLA-8 protein as an immunogen, or by using fragments of human CTLA-8 protein. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Additional peptide immunogens may be generated by replacing tyrosine residues with sulfated tyrosine residues. Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, J.Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, et al., FEBS Lett. 211, 10 (1987).

Neutralizing or non-neutralizing antibodies (preferably monoclonal antibodies) binding to human CTLA-8 protein may also be useful therapeutics for certain tumors and also in the treatment of conditions described above. These neutralizing monoclonal antibodies are capable of blocking the ligand binding to the human CTLA-8 protein or mayh promote clearance of protein from the patient.

Because of their homology to human CTLA-8, rat CTLA-8 proteins, herpes CTLA-8 proteins and IL-17 proteins (the "human CTLA-8" of Golstein et al., supra) will also possess CTLA-8 activity as described above. As a result, rat and herpes CTLA-8 proteins and IL-17 proteins, as well as active fragments and variants thereof, can be used in preparation of pharmaceutical compositions and in methods of treatment as described for human CTLA-8. Rat and herpes CTLA-8 proteins, and active fragments and variants thereof, can be produced as described above using the polynucleotides (or fragments or variants thereof) described in SEQ ID NO:3 and SEQ ID NO:5, respectively. Rat and herpes CTLA-8 may also be produced as described in Rouvier et al., J. Immunol. 1993, 150, 5445-5456. CTLA-8 proteins of other

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species can also be used as described herein. cDNAs encoding rat CTLA-8 and herpes CTLA-8 were deposited with the American Type Culture Collection on July 6, 1995 and assigned accession numbers ATCC 69867 and ATCC 69866, respectively. IL-17 proteins may also be produced as described in Golstein et al., supra.

Because of its homology to IL-17, the human CTLA-8 (B18) proteins of the present invention may also share some activities with IL-17.

For the purposes of treatment or therapy, any of the proteins discussed or disclosed herein may be administered by *in vivo* expression of the protein in a mammalian subject. In such instances, a polynucleotide encoding the desired protein is administered to the subject in manner allowing expression in accordance with known methods, including without limitation the adenovirus methods disclosed herein.

Example 1

Isolation of Human CTLA-8 cDNA

A partial clone for human CTLA-8 was isolated from a cDNA library made from RNA isolated from stimulated human peripheral blood mononuclear cells. This partial was identified as "B18." B18 is sometimes used herein to refer to the human CTLA-8 of the present invention. Homology searches identified this partial clone as being related to the herpes and rat CTLA-8 genes. DNA sequence of this partial clone was used to isolate the full-length clone.

In order to isolate a full-length cDNA for B18, a directional, full-length cDNA library by standard means in the COS expression vector pMV2. The cDNA library was transformed into *E. coli* by electroporation. The bulk of the original transformed cDNA library was frozen in glycerol at -80°C. An aliquot was titered to measure the concentration of transformed *E. coli*. The *E. coli* were thawed, diluted to 76,000/0.1 ml in media containing ampicillin, and 0.1 ml was distributed into the wells of a microtiter dish in an 8 x 8 array. The microtiter dish was placed at 37°C overnight to grow the *E. coli*.

To prepare DNA for PCR, 20 µl aliquots of culture from each well were withdrawn and pooled separately for each row and column of eight wells, giving 16

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pools of 160 µl each. The *E. coli* were pelleted, resuspended in 160 µl of standard lysis buffer consisting of 10 mM TrisHCI pH8, 1 mM EDTA, 0.01% Triton X-100, and lysed by heating to 95°C for 10 minutes.

To identify which of the wells contained *E. coli* transformed with B18, PCR was performed first on the DNA preps corresponding to the eight columns. The PCR consisted of two sequential reactions with nested oligonucleotides using standard conditions. The oligonucleotides used for the PCR reaction were derived from the sequence of the partial B18 clone. They were:

B185: CACAGGCATACACAGGAAGATACATTCA (SEQ ID NO:7)

B183: TCTTGCTGGATGGGAACGGAATTCA (SEQ ID NO:8)

B18N: ATACATTCACAGAAGAGCTTCCTGCACA (SEQ ID NO:9)

The PCR conditions were 2.5 mM MgCl₂ and 95°C x 2 min for one cycle, 95°C x 1 min plus 68°C x 1 min for 30 cycles, and 68°C x 10 for one cycle. Each reaction was 20 μ l. The first reaction contained oligonucleotides B185 and B183 and 1 μ l of the DNA preparations. The second reaction contained oligonucleotides B183 and B18N and 1 μ l of the first reaction.

DNA preps that potentially contained a full-length B18 cDNA clone were identified by agarose gel electrophoresis on an aliquot of the second PCR reaction. A DNA band of the correct mobility was assumed to be derived from a B18 cDNA. Next the same sequence of PCR reactions and gel analysis was done on the DNA preps corresponding to the eight rows. The intersection of a row and a column identified well A2 as potentially containing B18, narrowing it down to the 76,000 E. coli originally seeded into that well.

To further purify the individual *E. coli* containing the putative full-length B18 cDNA clone, the concentration of *E. coli* in well A2 was measured by titering and plating dilutions of the well. Then 7600 *E. coli* were seeded into the wells of a second microtiter plate in an 8 x 8 array. The *E. coli* were grown overnight; wells were pooled, and DNA was prepared as described above. To identify which of these wells contained *E. coli* transformed with B18, sequential PCR reactions were performed

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essentially as described above. Agarose gel electrophoresis identified well B2 as potentially containing a B18 cDNA.

The *E. coli* containing this cDNA was further purified by seeding wells of a microtiter plate with 253 *E. coli* per well and proceeding as for the purification of the *E. coli* in well A2. Well C3 was identified as containing a putative full-length B18 cDNA clone. The exact *E. coli* was identified by plating the contents of the well onto bacterial culture media and then screening the *E. coli* colonies following established protocols. The probe for these hybridizations was a PCR fragment generated by doing a PCR reaction on the B18 clone using as primers the oligonucleotides described above (SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9). When a single colony was identified, DNA was prepared and sequenced by standard methods. Comparison of this sequence to the sequence of the original partial clone confirmed identity and that the isolated cDNA was full-length.

The full-length clone was deposited with the American Type Culture Collection on July 6, 1995 and assigned accession number ATCC 69868.

Example 2

Expression of Human CTLA-8

The full-length B18 clone for human CTLA-8 was transfected into COS cells which were then labelled with ³⁵S-methionine. An aliquot of conditioned medium from the transfected cell culture was reduced, denatured and electrophoresed on polyacrylamide gels. Autoradiographs of those gels are reproduced in Fig. 2. The band indicated by the arrow demonstrates expression of human CTLA-8.

Example 3

Inhibition of Angiogenesis by Human CTLA-8

The ability of human CTLA-8 to inhibit angiogenesis was examined in an angiostatic activity assay (endothelial cell proliferation assay). The assay was done in a 96 well plate. Primary human umbilical cells (HUVECs) were seeded to $2x10^3$ cells per well in EGM medium (Clonetics)/20% FCS and incubated at 37° C for 24 hr. The cells were then starved in M199 medium (GIBCO BRL) containing 10% charcoal

treated serum (M199-CS) for 48 hr at 37°C. Conditioned media containing B18 (human CTLA-8) was obtained from transfected COS or stably expressing CHO cells and 1:10, 1:50, 1:250, and 1:1250 dilutions prepared in M199-CS medium containing 100 ng/ml FGF. The dilutions of B18 were added to the starved cells and incubated for 72 hr at 37°C. The cells were then radiolabeled by [³H]-thymidine for 6 hr. Radiolabeled cells were washed with PBS and trypsinized for liquid scintilation counting. Results were plotted using Kaleidograph software. The results are shown in Fig. 3. In the figure, "Med" is the mock control, "B18" and "B18-1" were conditioned medium from two independent transfections of COS with DNA encoding human CTLA-8 (B18). IFNγ was used as a positive control angiostatic (i.e., angiogenesis inhibition) activity. These data demonstrate that human CTLA-8 (B18) inhibits angiogenesis.

Example 4

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Hematopoietic Activity of Human CTLA-8

The hematopoietic activity of human CTLA-8 (B18) expressed in vivo was examined by construction of a recombinant adenovirus vector.

The B18 cDNA in the expression plasmid Adori 2-12 B18 was driven by the cytomegalovirus(CMV) immediate early promoter and enhancer.

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The Adori 2-12 vector was created by addition of an SV40 origin and enhancer to a known adenovirus vector (Barr et al., Gene Therapy 1:51 (1994); Davidson et al., Nature Genetics 3:219 (1993)). The HindIII/BamHI fragment encoding the SV40 origin and enhancer was isolated from the pMT2 mammalian expression vector, blunted with Klenow and cloned into the NatI site (blunted with Klenow) of the Ad5 expression vector.

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The vector was derived by digesting pNOT-B18 cDNA with SalI, filling in the 5' overhang with Klenow to generate a blunt end and digesting with EcoRI to isolate the B18 cDNA. The blunted- EcoRI B18 fragment was inserted into the restriction sites EcoRV-EcoRI of the adenovirus vector Adori 2-12. The CMV-B18 expression cassette was located downstream of the SV40 origin and enhancer, and 0-1 map units of the left hand end of the adenovirus type 5(Ad5). The SV40 splice donor and

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acceptor were located between the CMV promoter and B18 cDNA. Following the insert was SV40 poly A site, 9-16 map units of Ad5 and the puc 19 origin.

A recombinant adenovirus was generated by homologous recombination in 293 cells. AscI linearized Adori 2-12 B18 and ClaI digested AdCMVlacZ were introduced into the 293 cells using lipofectamine. Recombinant adenovirus virus was isolated and amplified on 293 cells. The virus was released from infected 293 cells by three cycles of freeze-thawing. The virus was further purified by two cesium chloride centrifugation gradients and dialyzed against PBS 4°C. Following dialysis of the virus glycerol was added to a concentration of 10 % and the virus was stored at - 70 °C until use. The virus was characterized by expression of the transgene, plaque forming units on 293 cells, particles/ml and Southern analysis of the virus.

A single dose of 5 X 10 ¹⁰ particles of recombinant adenovirus encoding B18 was injected into the tail vein of male C57/bl6 mice, age 7-8 weeks. Control mice received an adenovirus encoding B-galactosidase. Four mice from each experimental group were killed on day 7 and 14. Blood was collected and automated hematologic analysis was performed using a Baker 9000. Differential counts were performed on blood smears. Tissue was harvested, fixed in formalin, and stained with hematoxylin and eosin for histopathology. In the first set of experiments, serum and tissues were analyzed 7 and 14 days post injection. A slight increase in peripheral platelet counts were observed. The animals that received B18 exhibited a slight increase in spleen size. Macroscopic analysis of the spleen showed an increase in splenic extramedullary hematopoiesis on day 7 compared to the control. These results showed a hematopoietic growth activity associated with B18.

In a second set of experiments 5 X 10 ¹⁰ particles of recombinant adenovirus encoding B18 were injected into the tail vein of male C57/bl6 mice, age 17-18 weeks. Control mice received an adenovirus encoding B-galactosidase. Blood samples were collected via retro-orbital sinus on days 2, 5, 7, 10, 14, and 21. The hematologic analyses were performed on the Baker 9000 automated cell counter with murine-specific settings. Analyses included WBC, RBC, HCT and PLT counts. Blood smears were prepared and stained with Wright-Geimsa for WBC differentials based on a 100 cellcount. Reticulocytes and reticulated platelets were quantitated using flow

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cytometry. Four mice from each group were killed on days 7, 14, and 21. In addition to peripheral blood analysis, serum was collected via cardiac puncture for quantitation of systemic Il-6 using a commercial kit (Endogen). Spleen and liver were collected for histopathology, spleen and bone marrow hematopoietic progenitors were quantitated, and bone marrow smears were prepared and stained with Wright-Geimsa for cell counts.

Administration of adenovirus encoding B18 resulted in a marked increase in peripheral blood neutrophils and WBC (Fig. 4). Maximum increases in neutrophils were observed at day 5 and day 7. The control mice showed little difference at day 5 and day 7. Peripheral blood neutrophils were similar in the control mice and mice that received B18 at day 21. In both the B18 and control groups an increase in white blood cells was also observed. The mice that received B18 had a greater increase in WBC between day 2 and day 7. By Day 21 a more pronounced increase was observed in the B-gal group. No other changes in cellular chemistries were observed (Table I).

Bone marrow cellularity was calculated from pooled femurs in each group (Table III). No significant differences were observed in either group. No significant changes were observed in bone marrow hematopoietic progenitors from day 7, 14, and 21. The CFU-GM, BFU-E and CFU-MEG in the B18 mice were similar to the B-gal control (Table II).

Administration of the adenovirus encoding B18 resulted in an increase in CFU-GM (myeloid) and BFU-E (erythroid) progenitors in the spleen compared to animals that received the B-gal virus on day 7. The increase in progenitors in the B18 mice was 11-fold in CFU-GM and a 52-fold in BFU-E (Table II). There was a 2-fold increase in CFU-MEG at day 7 for the B18 mice. By day 21 no significant differences were observed in splenic CFU-MEG or BFU-E between the groups (Table II). A 3-fold decrease in CFU-GM was observed in mice that received adenovirus encoding B18. A slight increase in spleen size at day 7 was observed in the B18 group. This is consistent with an increase in splenic cellularity. By day 14 and day 21 spleen weights were similar to the control group (Table III). Macroscopic analysis of the spleen showed an increase in splenic extramedullary hematopoiesis of the B18 mice on day 7 compared to the control.

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The bone marrow myeloid: erythroid ratios (Table IV) suggest a granulocytic hyperplasia with a possible erythroid hypoplasia in mice that received adenovirus B18 on day 7. By day 21 the ratio in the B-gal group was higher. No changes were observed in the IL6 serum levels.

These results show a hematopoietic activity associated with the administration of adenovirus encoding B18 (human CTLA-8). Increases in neutrophils and white blood cells were observed at day 7 in animals that received B18 adenovirus. The data showed that B18 resulted in increase in splenic CFU-GM and BFU-E 7 days post administration compared to the control animals. Splenic extramedullary hematopoiesis on day 7 support that B18 exhibits a hematopoietic growth activity. These data suggest that B18 may mobilize early hematopoietic precursors.

Table I: Peripheral hematology for day 2, 5, 7, 10, 14, and 21.

~ AS4-409	16 (Platelo	1s) D	ay Z4-25	i-96.	_					bs Retics)	RCT I	PLT	APR A	De RPN
Group A	WBC	Nouts	ANC 1	Lymphs	ALC 10 - SAA	Eos	Monos	ABC 10-6/UL	%	10^6A/L	% 1	10^3/LL	% x	10 "3/vt.
B-Gal #1	±10^3A/L	40 K	2.16	54	2.92	0	6	10.88	3.65 2.04		48.0 56.6	836 900	11,94	99.82 90.90
B-Gal #2	7.4	25	1.85	65	4.81 3.54	3	7	12.34	3.25	0.37	51.6	894	9.77	87.34
B-Gal #3 B-Gal #4	6.8	40 23	2.72	52 64	5.63	i	12	12.00	2.55		54.8	840	10.63	89.29
AYG	7.1	32.0	2.19	50.0	4,22	1.5	7.8 1.4	11.62 0.33	2.68 0.36	0.23	52.6	17	0.48	2.76
BIÁ DI	11.4	59	6.73	31	3.53	10.8	0 1:7	11.15	4.88	0.54	52.4	1242	14.92	185.31
B18 42	9.2	30	2.76	62	5.70	3	5	10.14	3.97 3.23	0.40 0.38	48.0 51.2	632	11.18	83.02
B18 #3	5.0	51 41	2.55	40 65	2.00 3.52	:	1 1	10.00	3.09	0.33	49.2	904	17.31	156.48
B18 #4	6.4 E.O	45.2	3.67	47.0	3.69	1.0	6.0	10.82	3.79	0.41	50.2 1.0	903 127	13.58	125.92 27.07
BEM	1.4	6.3	1.02	7.0	0.76	0.7	1.3 }	0.24	0.41	0,031	1.01	1811	123	
Study A54-4B5	B18 (Pjetel	ets) C		8-96.				anc I	Retics	No Retice	HCT	PLT	RPIt	Abs RPR
Gronb B	WBC	Marks	ANC 10°3/A	Lympha	ALC 10 *SArL	Eos .	Monos %	X1046A/L		x10~e/ut.	% 1	1043A4L	%	10 *3As.
B-Gel #1	x10^3A:L	14	1,06	78	5.93	3	-5	11.26	8.25	0.59	624	1082 994	15.51 17.57	167.62 172.66
B-G# #2	10.6	20 18	2.12 ·1.51	78 69	6.27	1 2	- 1	10.72	4.71 3.40	0.38	51.2	916	9.55	67,48
B-Gal #3 B-Gal #4	10.5	38	4.10	58	8.26	ō	4	10.22	6.21	0.63	47.0	1092	13.93	152.12
AVO	9.5	22.5	2.20	70.8	6.63 0.55	1.5	5.3 2.1	10.83	4.89	0.53	1.2	41	1.47	19.57
SEM	14.8	18	2.66	71	10.33	7 00	10	12.66	2.31	0.29	67.0	1204	7.57	91.14
B18 #1 B18 #2	142	37	5.25	53	7.53	2	8	8.80	3.32 4.12	0.33	44.6 65.6	888 1134	14.33 10.15	115.10
B18 #3	12.6	30 58	3.64	59 37	7.55 5.92	:	10	12.12	3.93	0.43	80.6	1166	15.75	183.65
B18 #4 AVG	16.0	25.8	6.26	\$5.0	7.88	1.0	1.3	11.41	2.42	0.30	62.0	1098	11.95 1.88	19.61
SEM	0.7	8.4	1,44	/.1	0.96	0.4	1.2	0.63	0.41	0.05				
Study A54-48	B18 (Plate	lets)	Day 74-3	30-96 <u>.</u>			-		5.4	TL - 6:	HCT	PLT 1	APIE .	Abe RPR
Group C	WBC		ANC	Lymphs	ALC 10 *3/uL	Eos %	Monos	RBC X1004A4		Abe Hetice	%	x1043AL	%	210 AZAL
B-Gel #1	15.2	14	210^3/L/L	69	10.49		15	11.04	8.54	0.39	50.8	862	12.45	107.41 154.61
B-Gal #2	14.0	12	1,68	81	11.34	0	7 12	11.36	5.05 5.42	0.57 0.59	52.6 49.6	1104 952	11.49	109.38
B-Gal #3	14.8	14	2.07 1.96	73	10,50	0.7	11.7	11.11	4.57	0.52	61.0	873	12.05	127.13
AYO		0.7	0.14	3.5	0.25	0.3	2.6	0,14	9.54	0.06	452	864	1.02	18.75
B18#1	19.4	33	8.40 8.91	62 53	12.03	0	5	10.14	8.05	0.57	43.5	1288	12.49	180.67
818 #2 818 #3	25.4	39 44	10.38	50	11.80	C	6	9.74	8.17	0.50	44.4	1076 1136	15.41 15.68	185.81
818 64	12.8	15	1.92	75	9,60	۰	10 7.3	9.54 8.72	6.25 · 6.10	0.60	44.2	1081	14.15	154.42
AVC		37.5	7.15		0.00		1 :-	0.15	0.76	0.07	0.4	88	0.87	18,19
	4 9 8	6.3	1.96	9.0	0.441	0.0	1.1	4,12						
(35)		1	1,96		- 441	0.0	1	1						
Study A54-48.	_816 (Plat	eleta)	Day 10_1	5-3-06,	ALC	Eos	Monos	RBC	Retics	Abs Retics	нст	·PLT	RPR	Abe RPR
	B16 (Plat	Heuts	Day 10_I ANC x10^3Ad	5-3-96, Lymphs	VIC NIC	Eos %	Monos	RBC	Retics	Abs Retice	HCT %	PLT x10^3A4L	RPR %	236.52
Study A54-48. Group Å	B16 (Plate WBC x10^3/4	Hersta Hearta X	Day 10_1 ANC x10^2Ad 3.16	5-3-96, Lymphs 59	ALC ±10 *2/uL 12.83	Eoa %	Monoe y	RBC ×10^6AL 10.22	Retics % 12.41 6.00	Abs Retics x10^6AA. 127 0.63	46.8 48.8	1460 1460 1128	16.20 14.48	236.52 163.53
Study A54-48.	818 (Plate V/BC x10^3/4 18.6 13.2	Heuts	Day 10_I ANC x10^3Ad	5-3-96 Lymphs % 69 79 74	ALC x10 *3/ut. 12.83 10.43 14.60	E08 %	11	HBC x10^6A4 10.22 10.48 10.72	Retics % 12.41 6.00 6.25	Abs Retics x10^6AA. 1.27 0.63 0.67	46.8 46.8 49.4	x10^3A4. 1460 1128 1338	% 16.20 14.48 18.58	236.52
Study A54-4B. Group A B-Gal #1 B-Gal #2 B-Gal #3 B-Gal #4	810 (Plan V/BC x10*3A/1 18.6 13.2 19.6 18.6	Heats 17 18 16 21	Day 10_1 ANC x10^3Ad 3.16 2.11 3.14 3.91	5-3-96 Lymphs % 69 79 74 72	ALC x10 *3/ut. 12.83 10.43 14.60 13.39	Eos %	11 4	RBC x10^6A4 10.22 10.40 10.72	Retics % 12.41 6.00	Abs Retice x10^6AA. 1.27 0.63 0.67 0.79	48.8 48.8 49.4 40.4-	x10^3AAL 1460 1126 1338 1068 1249	96.20 14.48 18.50 14.25 18.40	206.52 163.53 221.64 153.26 153.26
Study A54-4B. Group A B-Gal #1 B-Gal #2 B-Gal #4 AV	816 (Plan WBC x10*3Ad 18.6 13.2 19.6 18.6 17.0	Houses 17 18 15	Day 10	5-9-06, Lymphs 56 79 74 72 73.5	ALC x10 *3Art. 12.63 10.43 14.60 13.39 12.79 6.85	E08 %	11 4 10 4 7.3	RBC x10~6A/L 10.22 10.48 10.72 10.44 10.47 0.10	Retics % 12.41 6.00 6.25 7.59 8.06 1.49	Abs Retics x10^6AA. 1.27 0.63 0.67 0.79 0.84 0.15	46.8 48.8 49.4 48.4- 48.4- 64.A	x10^3A.4. 1460 1128 1338 1068 1249	% 16.20 14.48 18.50 14.25 18.40 0.58	276.52 163.53 221.64 163.74 163.74 20.78
Study A54-48. Group A B-Gai #1 B-Gai #2 B-Gai #2 B-Gai #4 AVV SEI B16 #1	B10 (Plant VIBC x10*2Ad 18.6 13.2 18.6 18.6 17.4 14.2	Hessia % 17 18 15 15 17.5 1.2 133	Day 10 1 ANC x10-2Ad 3.16 2.11 3.14 3.91 9.05 6.37 4.69	5-9-06, Lymphs %. 69 79 74 72 7 73.5 7 2.1	ALC 12.63 10.43 14.60 12.39 12.79 6.86 7.95	Eoa %	11 4 10 4 7.3	RBC x10^4/4 10.22 10.48 10.72 10.44 10.47 0.10	Retics % 12.41 6.00 6.25 7.59 8.06	Abs Retice x10^6AA. 1.27 0.63 0.67 0.79	48.8 48.8 49.4 48.4- 48.4- 0.8 39.2 42.0	x10^3Ad. 1460 1128 1338 1068 1249 91 1760 1104	% 16.20 14.48 18.50 14.25 18.40 0.58 14.49	#10 *3AA 236.52 163.53 221.84 153.26 193.74 20.78 255.02 208.44
Study A54-48. Group A B-Gai #1 B-Gai #2 B-Jai #3 B-Gai #4 AV(SEI B16 #1	B18 (Plant WBC x19*3Ad 18.8 13.2 18.6 18.6 17.4 14.2 17.6	Hersta 17 16 15 15 21 17.5 1.3 3.3	Day 10	5-3-06, Lymphs % 69 79 74 72 73.5 7 2.1	ALC x10 *2/ut 12.63 10.43 14.60 13.39 12.79 6.85 7.95 10.03	E08 %	11 4 10 4 1.1 6 7	RBC x10^44.4. 10.22 10.48 10.72 10.44 10.47 0.10 8.70 9.04 4.74	Retics % 12.41 6.00 6.25 7.59 8.06 1.49 11.97 9.46 16.77	Abs Retics x10^4As. 1,27 0,63 0,67 0,79 0,94 0,15 1,04 0,86 0,79	46.8 46.8 49.4 48.4- * 48.4 0.8 39.2 42.0 22.4	x10^3AdL 1460 1128 1338 1088 1249 91 1760 1104 894	% 16.20 14.48 18.50 14.35 18.40 6.58 14.49 15.85 29.19	#10 *3A4, 226.52 163.53 221.84 153.26 193.74 20,78 255.02 206.44 200.96
Study A54-48. Group A B-Gai #1 B-Gai #2 B-Gai #2 B-Gai #4 AVV SEI B16 #1	B10 (Plant WBC x10~3Ad 18.8 13.2 19.6 17.4 17.2 17.6 16.2 14.2 17.6 16.2 14.2	Notes Notes	Day 10_1 ANC x10^2Ad 3.16 2.11 3.14 2.91 3.61 6.37 4.69 6.16 6.12 3.55	53-96, Lymphs 69 79 74 72 73.5 7 2.1 56 57 57 68	ALC 12.63 10.43 14.60 12.79 6.85 7.95 10.03 8.23 8.37	Eos 96	11 4 10 4 7.3 1.1	RBC x10^44,4 10.44 10.47 9.10 8.70 9.04 4.74 9.30	Retics % 12.41 6.00 6.25 7.59 8.06 1.49 11.97 9.46 16.77 9.83	Abs Retics x10^6A4. 1.27 0.63 0.67 0.79 0.84 0.15 1.04	48.8 48.8 49.4 48.4- 48.4- 0.8 39.2 42.0	x10^3/44 1460 1128 1338 1068 1249 91 1760 1104 894 1416	% 16.20 14.43 18.58 14.25 18.40 0.58 14.49 18.83 20.19 16.81	#19 *3A4, 226.52 163.53 221.84 153.26 193.74 20.78 255.02 206.44 200.96 216.03 240.61
Shudy AS4-48. Group A B-Gal #1 B-Gal #2 B-Gal #2 B-Gal #3 B-Gal #4 AV(BEI #1 B16 #1 B16 #2 B18 #3 B18 #4	B16 (Plate WBC 18.6 18.6 18.6 18.6 18.6 17.4 14.2 17.6 16.2 14.2	Heusta 5. 17 18 15 15 17 18 15 12 1 17 1 18 15 12 1 17 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Day 10_1 ANC x10*SAd 3.16 2.11 3.14 2.91 3.03 6.32 4.69 6.16 6.32 3.55	6-3-06, Lymphs % 69 79 74 72 73.5 7 2.1 56 57 57 66 8 \$9.0	ALC 12.63 10.43 14.60 12.79 6.85 7.95 10.03 8.23 8.37	Eos 96	11 4 10 4 7.3 1.1 7	RBC x10^44,4 10.44 10.47 9.10 8.70 9.04 4.74 9.30	Retics % 12.41 6.00 6.25 7.59 8.06 1.49 11.97 9.48 16.77 9.83	Abs Redics x10^6A4. 127 0.67 0.87 0.79 0.84 0.15 1.04 0.86 0.79 0.92	46.8 48.8 49.4 48.4- 98.3 39.2 42.0 22.4 42.0	x10^3AdL 1460 1128 1338 1088 1249 91 1760 1104 894	% 16.20 14.48 18.58 14.35 18.40 6.58 14.49 18.85 29.19	#10 *3A4_ 226.52 161.53 221.64 163.26 193.74 20.78 255.02 206.44 200.86
Study A54-48. Group A B-Gal 41 B-Gal 62 B-Gal 64 AV(BEI 611 B-18 62 B-18 44	B16 (Plate ViBC x10°3Ani 18.8 18.6 18.6 18.6 17.6 18.6 17.6 14.2 17.6 18.2 18.2 18.2 18.2 18.2 18.2 18.2 18.2	siets) Heuts 9,4 17 18 15 21 17.5 1.2 33 35 35 25 33.6 2.5 8 2.5 8 2.5	Day 10 ANC. #10-24d 3.16 2.11 3.16 2.21 3.01 3.01 6.37 4.09 6.16 6.32 3.55 6.10 6.60	5-3-96, Lymphs %. 69 79 74 72 73.5 2.1 56 57 68 59.0 6 2.1	ALC 210 *3Art 12.83 10.43 14.60 12.79 6.85 7.95 10.03 8.23 8.23 8.15	Eoa 96	11 4 10 4 7.3 1.1 7	HBC x10*474 10.22 10.48 10.72 10.44 1 10.47 9.10 8.70 9.04 4.74 9.30 7.85	Retics % 12.41 6.00 6.25 7.59 8.06 1.49 11.97 9.46 16.37 9.30 12.04	Abs Retics x10°6A4. 127 0.63 0.67 0.79 0.84 0.15 1.04 0.96 0.79 0.92	\$46.8 48.8 49.4 48.4- 68.0 39.2 42.0 22.4 42.0 36.4 4.7	1460 1460 1126 1338 1338 1088 1249 91 1760 1104 894 1416 1294	% 16.20 14.48 18.50 14.25 18.40 0.58 14.49 16.83 20.19 16.81 19.84 3.24	#10 - 3A4 226.52 163.53 221.64 153.26 163.74 20.78 255.02 206.44 200.96 216.03 240.61 11.77
Shudy A54-48, Group A B-Gal 41 B-Gal 52 B-Jal 63 B-Gal 64 B-Gal 64	B16 (Plant) WBC 18.6 18.6 18.6 18.6 17.3 14.2 17.3 16.2 14.2 14.2 0 16.2		Day 10	5-3-96, Lymphs 69 79 74 72 73.5 7 2.1 56 57 57 68 8 59.0 6 2.1	ALC x10 *2Ast 12.63 10.43 14.60 12.39 12.79 6.85 7.95 10.00 8.23 8.37 8.15 0.43	Eoa 96	11 4 10 4 7.3 1.1 7	RBC x10^SA4. 10.22 10.44 10.72 10.44 70.10 8.70 9.04 4.74 9.30 7.25 10.44 7.4 9.30 7.25 10.45 7.25 10.45 7.25 10.45 7.25 10.45 7.25 7.25 7.25 7.25 7.25 7.25 7.25 7.2	Retics % 12.41 6.00 6.25 7.59 8.06 1.47 9.46 10.77 9.83 12.04 1.87	Abs Retics x10*6/A. 127 0.53 0.57 0.79 0.15 1.04 0.05 0.79 0.90 0.90	46.8 48.8 49.4 48.4- 98.8 39.2 42.0 22.4 42.0 36.4	1460 1460 1126 1338 1338 1068 1249 91 1760 1104 894 1415 1294	% 16.20 14.48 18.58 14.35 18.40 0.58 14.49 18.65 20.19 16.81 19.84 3.24	#10 *3A4, 226.52 163.53 221.64 183.26 193.74 203.8 255.02 206.44 260.96 216.03 240.61 11.77
Shudy A54-48, Group A B-Gal 11 B-Gal 22 B-Jail 63 B-Gal 64 AV(B16 92 B18 92 B18 92 B18 94 AV SELECT A54-48 Group B	B16 (Plate 18.6 18.6 18.6 18.6 18.6 18.6 18.6 18.6		Day 10 ANC x10°3Ad 3.16 2.11 3.14 3.91 3.03 4.69 6.32 3.55 6.32 3.55 0.05	5-90, Lymphs % 69 79 74 72 73.5 7 2.1 56 57 57 68 59.0 8 59.0 8 Lymphs L %	ALC rip *Aut 12.83 10.43 14.60 12.39 12.79 6.85 7.95 10.00 9.23 6.35 6.45	Eos %	11 4 10 4 7,3 1,5 7 3 8,0 1,1	RBC 10-45AL 10-22 10-44 10-47	Retics % 12.41	Abs Retics x10*6/A. 127 0.53 0.57 0.79 0.15 1.04 0.05 0.79 0.90 0.90	46.8 48.8 49.4 48.4- 48.4- 84.A 0.8 39.2 42.0 22.4 42.0 34.4- 4.7	1460 1460 1128 1338 1068 1249 91 1700 1104 894 1416 1294 189	% 16.20 14.48 18.50 14.25 18.40 0.58 14.49 16.83 20.19 16.81 19.84 3.24	#10 -3A4 236.32 163.33 221.84 153.28 153.28 153.28 20.78 255.02 206.44 200.96 216.03 240.61 11.77
Sharly A54-48. Group A B-Gai #1 B-Gai #2 B-Jai #3 B-Gai #4 AV: B16 #1 B18 #3 B18 #4 Study A54-48 Group B B-Gai #1	S18 (Plate Plate	Heats Heat	Day 10 ANC x10*3Ad 3.16 2.11 3.14 2.91 3.6 6.32 3.55 6.32 3.55 6.32 3.65 6.32 6.32 6.32 6.32 6.32 6.32 6.32 6.32	5-90, Lymphe 99 79 74 72 73.5 7 66 57 66 2.1 5-7-96. Lymphe 4 92 74 74 72 73.5 7 66 57 75 766 8 75 766 75 75 766 75 75 75 75 75 75 75 75 75 75 75 75 75	ALC 10 *20.4 12.53 10.43 14.60 13.39 12.79 6.85 7.95 10.00 9.23 9.37 9.15 0.43	Eos 46	11 10 4 7.3 1.1 1.5 7.3 8 8 1.1 1.5 7.3 8 8 1.1 1.5 7.3 8 8 1.1 1.5 7.5 7.5 7.5 7.5 7.5 7.5 7.5 7.5 7.5 7	RBC 10-464 10-22 10-46 10-72 10-46 10-72 10-46 10-72 10-47 10-47 10-47 10-47 10-47 10-47 10-48 1	Retics % 12.41 4.00 4.25 7.59 8.06 1.49 14.97 9.46 16.77 9.23 12.04 1.87	Abs Retics x10°4A4. 1.27 0.67 0.79 0.84 0.16 1.04 0.82 0.92 0.82 0.92 0.95 Abs Retics x10°4A4. 0.65 0.77	46.6 48.8 49.4 48.4 9.6 39.2 42.0 22.4 42.0 34.4 4.7	100-304 1460 1128 1338 1088 1088 1249 91 1760 1104 1418 1294 1418 1294 1418 1294 1418 1294 1418 1294 1418	% 18.20 14.48 18.59 14.25 18.40 0.58 14.49 18.65 20.19 16.81 19.84 3.24 RPR %	#10 - 3A4_ 236.12 163.33 221.84 163.34 163.34 20.378 255.02 206.44 260.96 240.81 11.77
Study A54-48, Group A B-Gai #1 B-Gai #2 B-Gai #4 A56 B16 #1 B16 #2 B18 #3 A54 Study A54-48 Group B B-Gai #1 B-Gai #1	818 (Plate 13.2 13.5 13.2 13.5 13.5 13.5 13.5 13.5 13.5 13.5 13.5		Day 10	5.3-96, Lymphs 96, 69, 79, 74, 72, 73, 5, 75, 56, 57, 56, 68, 58, 58, 58, 58, 58, 58, 58, 58, 58, 5	ALC 12.03 12	Eos %	11 10 4 10 4 7.3 1.1 6 7 3 8 8 1,1 1,0 1,0 1,0 1,0 1,0 1,0 1,0 1,0 1,0	RBC 110-22 10-48 10-72 10-48 10-72 10-48 10-72 10-48 10-72 10-48 10-72 10-48 10-72 10-74 10-74 10-74 10-74 10-74 10-74 10-74 10-74 10-74 10-74 10-74 11-74 1	Retics \$ 12.41	Aba Retice 127 0.651 0.57 0.79 0.55 0.55 0.55 0.55 0.56 0.75 0.96 0.96 0.96 0.96 0.96 0.96 0.96 0.96	46.6 48.8 49.4 49.4 49.4 49.4 49.6 99.2 42.0 22.4 42.0 38.4 4.7 4.7 50.8 50.8 50.8 52.8	100-204. 1460 1128 1338 1088 1249 91 1760 1104 834 1415 1294 189 PLY 1109-204 1360 1818 1298	% 16.20 14.48 18.50 14.45 18.40 0.58 14.49 18.65 20.19 16.81 19.84 3.24 RPR %	#10 - 3AA. 236.12 163.53 221.84 153.24 153.24 153.24 20.78 255.02 206.44 250.96 240.81 11.77 Abs RPR ×10 - 3AA. 150.01 132.19 93.53 163.04
Shary A54-48, Group A B-Gal #1 B-Gal #2 B-Gal #2 B-Gal #3 B-Gal #3 B-Gal #4 AV SE Shary A54-48 Group B B-Gal #2 B-Gal #4	B10 (Plate VISC VISC VISC VISC VISC VISC VISC VISC		Day 10 L ANC x10-3Ad 3.184 2.11 3.14 3.21 3.81 4.89 6.16 6.16 6.16 6.16 0.85 0.87 ANC x10-34 ANC x10-34 5.20 5.20 5.20 5.20 5.20 5.20 5.20 5.20	5-3-95, Lymphre 979 74 72 77.5.57 2.1 56 55 57 66 8 \$9.0 6 2.1 5-7-95, Lymphre 4 56 56 56 57 57 66 59.0 6 5	ALC 12.63 10.43 10.43 14.60 13.39 12.79 6.85 7.95 10.00 9.37 8.15 0.43 13.19 13.17 13.17 13.14 14.40 10.26	Eco (1) 1 1 2.1 1.5 (1) 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	11 10 10 11 10 10 10 10 10 10 10 10 10 1	RBC 10.22 10.46 10.72 10.44 10.47 0.10 6.70 9.54 4.74 9.30 7.85 1.08 10.92 11.38 10.92 11.38 10.92 11.38 0.10 10.92 11.38 0.10 10.92 11.38 0.10 10.92 11.38 0.10 10.92 11.38 0.10 10.92 10.92 11.38 0.10 10.92 10.	Retics 12.41	Abe Redice x10°44A. (2.51 0.57 0.77 0.79 0.94 0.15 0.79 0.90 0.90 0.90 0.90 0.90 0.90 0.90	** 48.8 48.8 49.4 ** 44.4 ** 44.4 ** 44.0 39.2 42.0 22.4 42.0 38.4 4.7 ** HCY %** 50.8 50.8 52.6 43.0 43.0 43.4 ** 44.4 47.0	100-3/44 1460 1128 1338 1038 1048 11760 1104 834 1418 1294 1418 1294 1390 1360 1360 1360 1360 1487 1488 1489 1489 1489 1489 1489 1489 1489	% 16.20 14.48 18.25 14.25 14.25 14.49 18.65 29.19 16.81 19.84 3.24 RPR % 11.03 8.18 7.30 10.05 9.15	#10 *3A4 236.52 161.53 221.84 153.26 153.26 153.26 203.44 250.96 240.61 11.77 Abs RPR #19 *3A4 150.01 132.19
Sharly A54-48, Group A B-Gai #1 B-Gai #2 B-Jai #3 B-Gai #3 B16 #1 B16 #2 B18 #3 B18 #3 B18 #4 Security A54-48 Group B B-Gai #1 B-Gai #1 B-Gai #1 B-Gai #1 B-Gai #1 B-Gai #1	B16 (Plant 18.6 18.6 18.6 18.6 18.6 18.6 18.6 18.6	Houts House Hous	Day 10 ANC ANC	5-3-95, Lymphs 979 79 74 72 73.5 7 2.1 56 57 56 66 8 59.0 6 7.4 4 66 99.5 71 15 15 15 15 15 15 15 15 15 15 15 15 15	ALC 12.53 10.43 14.50 12.59 12.79 0.85 7.95 10.01 10.0	Ecos %	7.3 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0	RBC 10.22 10.46 10.72 10.44 10.70 10.44 10.70 10.44 10.70 10.44 10.70	Retics % 12.41	Abe Redice 10-57 0.57 0.57 0.57 0.57 0.57 0.57 0.57 0.	**************************************	100-3A4. 1460 1128 1338 1338 1008 1249 91 1760 1104 894 1415 1284 1415 1284 1415 1284 1416 1360 1616 1286 1672 1487	16.20 14.48 14.48 14.45 14.45 14.49 16.65 20.19 16.65 20.19 16.81 19.84 3.24 RPR 5.00 10.05 8.18 7.30 10.05 8.18	#10 - 3AA. 236.12 163.53 221.84 153.24 153.24 153.24 20.78 255.02 206.44 250.96 240.81 11.77 Abs RPR ×10 - 3AA. 150.01 132.19 93.53 163.04
Shary A54-48, Group A B-Gal #1 B-Gal #2 B-Gal #2 B-Gal #3 B-Gal #3 B-Gal #4 AV SE Shary A54-48 Group B B-Gal #2 B-Gal #4	B18 (Plate VISC VISC VISC VISC VISC VISC VISC VISC	House Hous	Day 10. L ANC ANC 2.10 3.18 2.11 3.14 3.19 3.20 6.10 6.32 3.52 6.10 6.32 3.52 6.10 6.1	5-3-06. Lymphs 69 79 74 72 57 73.5 7 2.1 56 2.1 5-7-06. Lymphs 4 74 66 90 57 13 71 16 77 8 8 8 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	ALC 110 *2A/L 12.63 10.63 14.60 13.39 12.79 6.85 7.95 10.03 8.23 8.23 8.23 8.21 ALC 13.17 13.17 13.17 13.16 14.40 10.26 20.26 1	Eco	11 4 10 4 1.0 1.1 1.0 1.1 1.0 1.1 1.1 1.1 1.1 1.1	RBC 10.22 10.46 10.27 10.44 10.47 10.47 10.47 10.44 10.47 10.47 10.47 10.47 10.47 10.47 10.47 10.48	Retics % 12.41 4.00 4.05 7.50 11.57 9.40 11.57 9.40 11.57 8.51 12.04 1.57 8.67 7.07 4.41 7.82 14.6.74	Abe Redice x10°444. (0.51 0.87 0.79 0.94 0.15 0.79 0.90 0.90 0.90 0.90 0.90 0.90 0.90	** 48.8 48.8 49.4 ** 48.4 ** 48.4 ** 48.4 ** 48.4 ** 48.4 ** 42.0 ** 22.4 42.0 ** 38.4 4.7 ** 50.8 \$2.8 \$2.8 \$3.8 \$3.8 \$3.8 \$4.8 \$	100-3/44 1460 1128 1338 1038 1048 11760 1104 834 1418 1294 1418 1294 1390 1360 1360 1360 1360 1487 1488 1488 1489 1489 1489 1489 1489 1489	16.20 14.48 18.25 14.43 14.35 14.49 16.83 20.19 16.81 19.84 3.24 RPR 5. 11.03 8.18 7.39 10.05 8.18 10.05 8.18 10.05 8.18 10.05 8.18 10.05 8.18 10.05 8.18 10.05 8.18 10.05 8.18 10.05 8.18 10.05 8.18 8.18 10.05 8.18 8.18 8.18 8.18 8.18 8.18 8.18 8.1	170 *344 126.52 181.33 121.34 183.26 183.26 183.26 208.44 209.96 208.44 209.96 208.41 11.77 Abs RPR 112.79 182.01 122.19 193.53 183.01 122.19 193.53 183.01
Shary A54-48, Group A B-Garl #1 B-Garl #2 B-Garl #2 B-Garl #3 B-Garl #3 B-Garl #4 B18 #2 B18 #3 B18 #3 B18 #3 B18 #3 B18 #4 B18 #3 B18 #4 B18 #3	B18 (Plate VISC VISC VISC VISC VISC VISC VISC VISC	Hearts H	Opy 10 ANC ANC ANC ANC ANC ANC ANC 2.11 3.14 3.21 3.05 6.37 4.69 6.10 6.35 6.10 6.35 0.87 ANC	5-3-95, Lymphs 979 79 74 72 73.5 7 2.1 56 57 56 66 8 59.0 6 7.4 4 66 99.5 71 15 15 15 15 15 15 15 15 15 15 15 15 15	ALC 110 *2Art 12.63 10.43 14.60 13.39 12.79 6.85 7.95 10.03 8.27 8.15 0.43 ALC XION 13.17 13.46 14.40 10.26 10.27 1	Eco	11 4 10 4 11 11 11 11 11 11 11 11 11 11 11 11 1	RBC 10.42 10.42 10.42 10.42 10.42 10.42 10.47 10	Retics % 12.41 4.00 4.25 7.59 1.87 14.77 9.40 14.87 14.87 14.97 14.97 14.97 14.97 14.97 14.97 14.97 14.97 15.97 16	Abs Resides x10°444. (0.33 0.87 0.79 0.94 0.15 1.04 0.15 1.04 0.79 0.90 0.90 0.90 0.90 0.90 0.90 0.90	**************************************	1003 1460 1460 1460 1460 1460 1460 1460 1660	16.20 16.20 18.25 18.25 14.25 18.25 18.25 18.25 18.25 18.25 18.25 18.24 19.24 2.24 11.03 8.16 9.51 9.51 9.51 9.51 9.51 9.51 9.51 9.51	170 *344 126.53 121.84 183.35 123.54 183.56 183.56 200.56 200.56 200.56 200.56 200.56 200.56 111.77 Abs RPR (19.244 112.01 122.01 123.01 124
Shary A54-48, Group A B-Garl #1 B-Garl #2 B-Garl #2 B-Garl #3 B-Garl #3 B-Garl #4 B16 #1 B18 #2 Stary A54-48 Group B B-Garl #1 B-Ga	B10 (Plate VISC VISC VISC VISC VISC VISC VISC VISC	Newton	Day 10 1 ANC x10-31d 3-31d	5-3-95, Lymphs 76, 77, 77, 77, 77, 77, 77, 77, 77, 77,	ALC 110 *SA41 12.63 10.43 10.43 10.43 12.79 12.79 10.00 9.23 10.43 10.43 10.43 10.43 10.26	Eos 45	# # # # # # # # # # # # # # # # # # #	RBC 10.22 10.42 10.22 10.42 10.47 10	Retics % 12.41	Abe Redice 1107 0.51 0.51 0.51 1.04 0.05 0.55 0.55 0.55 0.55 0.55 0.55 0	**************************************	100-344	16.20 16.20 18.50 18.50 18.51 14.25 18.25 18.25 18.25 18.25 18.25 18.24 224 224 224 224 224 225 11.03 8.16 9.51 9.51 9.51 9.51 9.51 14.20 9.51 14.20 9.51 14.20 9.51 14.20 9.51 14.20 9.51 14.20 9.51 14.20 9.51 14.20 9.51 14.20 9.51 14.20 9.51 9.51 9.51 9.51 9.51 9.51 9.51 9.51	170 *344 126.53 121.84 183.35 183.76 183.76 255.02 256.04 250.96 240.81 240.
Sharty A54-48, Group A B-Gai #1 B-Gai #2 B-Jai #3 B-Gai #4 B18 #1 B18 #1 B18 #2 B18 #3 B18 #4 AV SCIENT A54-48 Group B B-Gai #1 B-Gai #2 B-Gai #2 B-Gai #2 B-Gai #2 B-Gai #1 B-Gai #2 B-Gai #1 B-Gai #2 B-Gai #1 B-Ga	816 (Plate Wiscons) 18.6 18.6 18.6 18.6 18.6 18.6 18.6 18.6		Opy 10 ANC	5-3-96, Lymphe	ALC 110 *3At1 12.53 10.43 14.60 13.39 12.79 8.35 7.95 10.05 9.37 8.15 0.43 13.17 13.17 13.17 13.17 13.17 13.42 14.40 10.28 10.29 10.	Ecos	11 14 14 15 15 15 15 15	RBC 10.22 10.42 10.22 10.42 10.47 10	Retics % 12.41 4.00 4.25 7.59 8.06 14.97 9.46 16.77 9.20 12.20 14.97 8.91 1.57 9.41 1.	Abs Redics x10*444. (27 0.83 0.87 0.96 0.94 0.15 0.99 0.92 0.90 0.90 0.90 0.90 0.90 0.90	**************************************	100-344	16.20 14.48 18.20 14.48 18.20 14.35 18.21 14.35 16.81 18.84 2.24 RPR % 11.03 8.18 7.30 10.05 11.03 8.18 7.30 10.05 11.03 8.18 9.31 14.29 15.25	#19 "AM 170 "AM 170 "AM 170 "AM 170 AM
Sharty A54-48, Group A B-Gai #1 B-Gai #2 B-Jai #3 B-Gai #3 B18 #1 B18 #3 B18 #3 Sharty A54-48, Group B B-Gai #1 B-G	B18 (Plate 1 18.6 1		Day 10 10-34 2.11 2.11 3.01 3.01 3.01 3.01 3.01 3.02 3.03 4.69 6.12 3.5 6.12 3.5 6.17 ANC X10-34 ANC X10-34 ANC X10-34 4.61 4.61 4.61 4.61 4.61 4.61 4.61 4.61 4.61 4.61 4.61 4.61 4.61 4.61	5-3-96, Lymphs	ALC 110 *3At1 12.53 10.43 14.60 13.39 12.79 8.35 7.95 10.05 9.37 8.15 0.43 13.17 13.17 13.17 13.17 13.17 13.42 14.40 10.28 10.29 10.	Ecos	11 4 7.3 1.1 6 7 3 8 8 1.1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	RBC 10.47 0.10 0.10 0.10 0.10 0.10 0.10 0.10 0.1	Retics % 12.41 4.00 4.25 7.59 8.06 14.97 9.46 16.77 9.20 12.20 14.97 8.91 1.57 9.41 1.	Abs Redics x10°444. (127 0.83 0.87 0.99 0.94 0.15 1.04 0.99 0.90 0.90 0.90 0.90 0.90 0.90 0	**************************************	100-344	16.20 16.20 18.50 18.50 18.51 14.25 18.25 18.25 18.25 18.25 18.25 18.24 224 224 224 224 224 225 11.03 8.16 9.51 9.51 9.51 9.51 9.51 14.20 9.51 14.20 9.51 14.20 9.51 14.20 9.51 14.20 9.51 14.20 9.51 14.20 9.51 14.20 9.51 14.20 9.51 14.20 9.51 9.51 9.51 9.51 9.51 9.51 9.51 9.51	#10 "3/4" 128.23 121.34 123.24 123.24 123.24 123.24 123.24 123.24 123.24 123.25 123.24 123.25
Shary A54-48, Group A Group A B-Gai #1 B-Gai #2 B-Gai #3 B-Gai #4 AVI B-Gai #4	818 (Plate 19 19 19 19 19 19 19 19 19 19 19 19 19	Noncia N	Osy 10! pto-234 2.16 2.16 2.16 2.17 3.07 6.02 6.02 6.02 6.02 6.02 6.02 6.02 6.03 6.02 6.03 6.03 6.04 6.02 6.03 6.03 6.04 6.04 6.04 6.04 6.04 6.04 6.04 6.04	5-3-06, Lymphs 76, 77, 77, 77, 77, 77, 77, 77, 77, 77,	ALC 10 *3Aut. 10 *3Aut. 10 *3 10 *3 14 *50 12 *39 12 *79 10 *15 10 *1	Ecos	11 4 7.3 1.1 6 7 3 8 8 1.1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	RBC 10.42 10.42 10.42 10.42 10.42 10.42 10.47 10	Retics % 12.24 4.00 4.25 7.25 7.25 16.77 9.20 16.77 9.20 16.77 9.21 16.77 9.21 16.77 9.22 16.77 9.23 16.77 9.24 17.62 18.62 18.62 18.62 18.62 18.62 18.62 18.62	Abs Redics x10°444. (27' 0.83' 0.87' 0.99' 0.94' 0.15' 1.04' 0.99' 0.90'	**************************************	1400 1400	16.20 16.20 16.20 16.25	#10 *344 128.25 128.25 123.31 123.44 123.26 123.74 123.74 123.74 123.74 123.75 123.76 133.76 1
Sharty A54-48, Group A B-Gai #1 B-Gai #2 B-Jai #3 B-Gai #3 B18 #1 B18 #3 B18 #3 Sharty A54-48, Group B B-Gai #1 B-G	B16 (Plate V MSC V		Day 10 10	5-3-95, Lymphs 6-9 7-9 7-2 7-7 7-2 7-7 7	ALC 10 - 3 ALC	Ease % 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	7.2 1.1 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5	RBC 10.42 10.42 10.42 10.42 10.42 10.42 10.47 10	Retics % 12.24 4.00 4.25 7.25 7.25 16.77 9.20 16.77 9.20 16.77 9.21 16.77 9.21 16.77 9.22 16.77 9.23 16.77 9.24 17.62 18.62 18.62 18.62 18.62 18.62 18.62 18.62	Abs Redics x10°444. (27' 0.83' 0.87' 0.99' 0.94' 0.15' 1.04' 0.99' 0.90'	**************************************	1400 1700	FL 10.05 1.05 1.05 1.05 1.05 1.05 1.05 1.0	#10 *344 196.33 193.33 193.34 193.24 193.24 193.24 193.24 293.26 240.61 240.61 240.61 11.77 Abs RPR 11.77 150.01 122.19 93.53 168.04 194.05
Shurty AS4-48, Group A B-Gail et	B 816 (Plant 18.6 18.6 18.6 18.6 18.6 18.6 18.6 18.6		Day 10. P. Control of the control of	5-3-06, Lymphs 7-4 7-7 7	ALC ir10 *3hul. 12.83 10.43	Eos %	# 6 6 7 3 8 6 5 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	RBC 10.47 9.50 1 10.87 9.50 1 10.87 9.50 1 10.87 9.50 1 10.87 9.50 1 10.87 1 10.87 9.50 1 10.87 1 10.87 9.50 1 10.87 1 10.87 9.50 1 10.87 1 10.87 9.50 1 10.87 1 10.87 9.50 1 10.87 1 10.87 9.50 1 10.87 1 10.87 9.50 1 10.87 1 10.87 9.50 1 10.87 1 10.87 9.50 1 10.87 1 10.8	Retics % 12.24 4.00 4.25 7.25 7.25 16.77 9.20 16.77 9.20 16.77 9.21 16.77 9.21 16.77 9.22 16.77 9.23 16.77 9.24 17.62 18.62 18.62 18.62 18.62 18.62 18.62 18.62	Abs Redics x10*444. (27 0.83 0.87 0.97 0.98 0.19 0.92 0.85 0.99 0.92 0.85 0.79 0.92 0.85 0.79 0.92 0.85 0.79 0.85 0.79 0.85 0.79 0.85 0.85 0.77 0.73 0.71 0.71 0.71 0.71 0.71 0.72 0.85 0.85 0.85 0.85 0.85 0.85 0.85 0.85	**************************************	1400 1700	FINAL PROPERTY OF THE PROPERTY	#10 *344 128.23 123.33 123.44 153.25 153.24 153.25 153.24 153.25 153.26 1
Sharly A54-48. Group A B-Gai e1 B-Gai e2 B-Gai e3 B-Gai e4 Stell e3 B-Gai e4 B	B 818 (Plate 1948) 18.6 13.2 19.6 18.6 13.2 19.6 18.6 18.6 19.6 19.6 19.6 19.6 19.6 19.6 19.6 19	Noncla N	Day 10 10	5-3-06, Lymphs 69 79 74 77 72.1 56 57 56 6 57 56 6 57 57	ALC 10 - 3 ALC	Ease % 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	7.2 1.1 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5	RBC 10.47 0.10 0.10 0.10 0.10 0.10 0.10 0.10 0.1	Retics % 12.41 4.00 4.25 7.59 8.06 14.97 9.46 16.77 9.30 12.04 1.47 8.07 8.07 8.41 7.62 7.67 8.41 1.53 4.99 8.57 8.41 1.53 4.99 8.57 8.41 1.53 4.99 8.57 8.41 1.53 8.57 8.57 8.57 8.57 8.57 8.57 8.57 8.57	Abs Restics x10*444. (27 c.83 c.84 c.95 c.84 c.95 c.85 c.97 c.96 c.96 c.96 c.96 c.96 c.96 c.96 c.96	**************************************	1400 1400	16.20 16.20 16.20 14.45 14.49 18.65 29.19 16.81 18.85 29.19 16.81 19.84 3.24 RPR % 11.03 8.16 9.51 14.29 15.79 16.65 14.29 15.79 16.65 14.29 15.79 16.65 14.29 15.79 16.65 14.29 15.79 16.65 14.65 16.	#10 *344 126.53 121.34 183.32 183.32 183.78 183.78 183.64 200.44 200.94 240.61 11.77 Abs RPR 710 *344 183.05 183.05 184.45 184.45 184.45 184.45 184.65 18
Shury AS4-48, Group A B-Gai #1 B-Gai #2 B-Gai #4 B-Gai #1	B 816 (Plant 18.6 18.6 18.6 18.6 18.6 18.6 18.6 18.6	Noncia N	Day 10 10-34 10 10-34 2.11 3.14 3.13 3.14 3.9 8.16 8.32 3.55 10 0.54 ANC x10-34 ANC x10-34 1.13 1.33 4.37 5.20 1.12 4.47 5.20 6.41 1.33 4.77 5.20 6.41 1.33 4.77 5.20 6.41 1.33 4.77 5.20 6.43 4.77 5.20 6.43 4.77 6.43 6	5-3-96, Lymphs 69 79 74 77 72.1 56 57 56 6 57 57 58 59 68 59 59 59 59 59 59 59 5	ALC 110 *3 ALC 110 *3 ALC 12.53 10.43 10.43 10.43 10.43 10.45	Eco	Monoco 10 10 10 10 10 10 10 1	RBC 10.47	Retics %. 12.04 (1.07 1.07	Abe Retice 1021 0.84 0.15 0.97 0.99 0.95 0.95 0.95 0.95 0.95 0.95 0.95	**************************************	1460 1760	RPR 9.51 14.55 14.55 14.55 14.55 14.55 15.	#10 *344 128.33 221.34 183.25 183.37 183.24 183.26 183.24 183.26 183.24 200.44 200.36 240.61 11.77 240.61 11.77 240.61 12.19 243.51 183.04 132.19 243.51 183.04 132.19 243.51 183.04 132.19 243.51 183.05 183.
Shurty AS4-48, Group A B-Gail #1 B-Gail #2 B-Gail #4	816 (Plant WBC 18.6	New Act New	Day 10. 10	5-3-06, Lymphs 7-4 7-7 7	ALC irlo *3Aul. 10.43	Eos % 3 1 1 2.2 2.3 1 1 1 2.2 2 2 2 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Monoco 10 10 10 10 10 10 10 1	RBC 10.47 0.10 0.10 0.10 0.10 0.10 0.10 0.10 0.1	Retics % 12.41 4.00 4.25 7.59 1.67 9.46 14.57 9.46 14.57 9.46 14.57 9.46 14.57 9.46 14.57 9.46 14.57 9.46 14.57 9.46 14.57 9.46 14.57 9.46 14.57 9.41 9.4	Abe Redice 210-244 (1974) (197	**************************************	1400 1700	RPR 9.51 14.05 1.05 1.05 1.05 1.05 1.05 1.05 1.05 1	#10 "3/4" 170 "3
Sharty A54-48, Group A B-Gai #1 B-Gai #2 B-Jan #2 B-Jan #3 B-Gai #1 B16 #1 B16 #2 Sharty A54-48 Group B B-Gai #1	B18 (Plate 19.6 18.6	New York	Day 10. 10	5-3-06, Lymphs 7-4 7-7 7	ALC 19.253 10.43 1	Eoo %	# # # # # # # # # # # # # # # # # # #	RBC 10.47 0.10 0.10 0.10 0.10 0.10 0.10 0.10 0.1	Retics %. 12.41 6.00 6.25 7.59 1.137 9.46 14.57 9.46 14.57 9.46 14.57 9.46 14.57 9.46 14.57 9.41 1.57 9.41 1.57 9.41 1.57 9.41 1.57 9.41 1.57 9.41 1.57 9.57 1.57 9.57	Abs Retics 10°494.4. (27°494.4. (28°494.4. (**************************************	1400 1400	RPR %. 14.09 18.59 18.59 18.59 18.59 18.59 18.59 18.59 18.59 18.50 18.51	#10 *344 128.33 221.34 183.25 183.37 183.24 183.26 183.24 183.26 183.24 200.44 200.36 240.61 11.77 240.61 11.77 240.61 12.19 243.51 183.04 132.19 243.51 183.04 132.19 243.51 183.04 132.19 243.51 183.05 183.
Shurty A54-48, Group A B-Gai #1 B-Gai #2 B-Gai #2 B-Gai #4 B-B #1 B16 #1 B16 #1 B16 #2 B18 #4 AV STUDY A54-48 Group A B-Gai #4 AN B18 #1 B-Gai #1	B18 (Plate Plate P	Noncta N	Day 10 10	5-3-96, Lymphs 69 79 74 77 56 6 57 56 6 57 57	ALC 10 *3 ALC 10 *3 ALC 10 *3 ALC 10 *3 ALC 10 *4 ALC	Eas % 0 1 1 1 2 1 2 0 2 1 1 1 2 0 0 3 3 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0	# # # # # # # # # # # # # # # # # # #	RBC 10.47	Retics	Abe Redice 10.24 (0.25 (0.27 (**************************************	1400 1700	RPR 7.01 14.00 1.00 1.00 1.00 1.00 1.00 1.00	#10 *344 #10 *344
Shurty A54-48, Group A B-Gai #1 B-Gai #2 B-Gai #2 B-Gai #2 B-Gai #4 B-	B16 (Plant VIEC NO. 18.6 18.	Hends 17 16 17 17 18 17 18 18 18 18	Day 10_1 pto-24 10_1 pto-24 10_2 pto-24 10	5-3-06, Lymphs 1-74 1-75	ALC 19.253 10.43 1	Eas % 0 1 1 1 2 1 1 1 2 0 2 1 1 1 1 2 0 0 3 1 1 1 1 0 0 0 1 1 1 1 1 1 1 1 1 1	# 10 11 12 11 12 12 12 12 12 12 12 12 12 12	RBC 10.47	Retics	Abe Resides 10-24 (**************************************	1460 128	## 16.20 14.49 14.55 14.49 16.85 14.49 16.85 20.19 16.85 20.19 16.85 16.81 7.29 10.05 8.18 7.29 10.05 8.18 9.51 14.29 15.79 16.65 8.44 11.65 8.44 11.65 7.60 8.37 10.45 7.60 7.60 7.60 7.60 7.60 7.60 7.60 7.60	#10 *344 1284
Shary A54-46, Group A Group A B-Gai #1 B-Gai #2 B-Jai #3 B-Gai #1 B18 #1 B18 #1 B-Gai #1	B 18 (Plant 19.6) 18.6		Day 10_1 pto-24 10 10 10 10 10 10 10 10 10 10 10 10 10	5-3-06, Lymphs 1-74	ALC 110 *3Ast 12.53 10.6	Eos %	# # # # # # # # # # # # # # # # # # #	RBC 10.47 0.10 0.10 0.10 0.10 0.10 0.10 0.10 0.1	Retics % 12.41 6.00 6.25 7.59 1.1.97 9.46 16.77 9.46 16.77 9.40 16.77 9.40 16.77 9.41 16.20 4.91 16.20 16	Abs Redics 119-744.4. (27) 0.83 0.87 0.99 0.94 0.15 1.04 0.15 0.90 0.90 0.95 Abs Redics 0.79 0.92 0.90 0.95 Abs Redics 0.73 0.73 0.73 0.73 0.73 0.73 0.73 0.73	**************************************	1400 1200	RPR 7.00 14.05 14.05 14.05 16.	#10 *344 128.23 129.14 152.25 152.31 152.24 152.24 152.24 152.24 152.24 152.24 152.25 152.37 153.38 153.37 153.38 153.37 153.38 153.37 153.38 153.37 153.38 153.37 153.38 153.37 1
Shary A54-48, Group A B-Gai #1 B-Gai #2 B-Gai #4 B-G	B 16 (Plate W 16C		Day 10_1 pt 10	5-3-06, Lymphs 1-74	ALC 10 *3 ALC 10 *3 ALC 10 *3 ALC 10 *3 ALC 10 *4 ALC 12 *5 ALC	Eos %	# # # # # # # # # # # # # # # # # # #	RBC 10.47	Retics % 12.41 6.00 6.25 7.59 1.1.97 9.46 16.77 9.46 16.77 9.40 16.77 9.40 16.77 9.41 16.20 4.91 16.20 1	Abs Redics 119-744.4. (27) 0.83 0.87 0.99 0.94 0.15 1.04 0.15 0.90 0.90 0.95 Abs Redics 0.79 0.92 0.90 0.95 Abs Redics 0.73 0.73 0.73 0.73 0.73 0.73 0.73 0.73	**************************************	1400 1400	RPR 9.51 14.05 14.	#10 *344 128.23 129.14 152.25 152.31 152.24 152.24 152.24 152.24 152.24 152.24 152.25 152.37 153.38 153.37 153.38 153.37 153.38 153.37 153.38 153.37 153.38 153.37 153.38 153.37 1

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Table II: Bone marrow and Splenic Hematopoietic Progenitors

	CFU	-MEG	CFU-GM		BFU-E	
Bone Marrow*	B-Gal	B18	B-Gal	B18	B-Gal	B18
Day 7	16.0 ± 3.5	15.7 ± 3.1	307 ±117	241±78	51 ± 19	25 ± 11
Day 14	10.7 ± 2.3	15.3 ± 1.2	233 ± 15	373±35	30 ± 10	60 ± 30
Day 21	5.7 ± 0.6	6.7 ± 3.1	170 ±	160±27	40 ± 10	27 ± 6
Spleen**						
Day 7	9.3 ± 1.6	19.5 ± 1.5	27 ± 3	298 ± 6	1.3 ± 1.2	68 ± 10
Day 14	9.7 ± 0.6	12.7 ± 0.6	267 ± 32	197 ±21	33 ± 6	10 ± 10
Day 21	17.0 ± 1.0	19.3 ± 2.5	187 ± 6	73 ± 15	23 ± 6	23 ± 6

Hematopoietic precursors were determined form pooled spleen and bone marrow samples from four animals in each group. For quantitation of CFU-GM and BFU-E, either 1 x 10⁴ bone marrow cells or 1 x 10⁵ spleen cells were added to complete alpha methylcelluose medium (0.9% methylcellulose in alpha medium, 30% fetal bovine serum, 1% bovine serum albumin, 10-4M 2-mercaptoethanol, 2 mM L-glutamine, 2% murine spleen cell conditioned medium, and 3 U/mL erythropoietin) and aliquoted into 35 mm tissue culture dishes in a final volume of 1.0 mL. Cultures were incubated for 7 days at 37°C, and 5% CO₂. Microscopic colonies were defined as clusters of 50 or more cells. For quantitaion of CFU-MEG, either 1 x 10⁵ bone marrow cells or 1 x 10⁶ spleen cells were added to complete alpha methylcellulose medium and incubated as described above. Megakaryocyte colonies were defined as a group of 3 or more cells.

^{*}Bone marrow progenitors are represented as mean ± sd number of colonies per 10⁵ cells.

^{**}Spleen progenitors are represented as mean ± sd number of colonies per 10⁶ cells.

Table III: Spleen Weights and Femur Cellularity

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Spleen Wt. (Mg)	B-Gal	B18	Femur Cellularity (x10 ⁶)	B-Gal	B18
Day 7	187 ± 19	224 ± 29	Day 7	28	23
Day 14	175 ± 13	170 ± 10	Day 14	28	27
Day 21	174 ± 21	151 ± 27	Day 21	28	26

Spleen weights were determined at time of sacrifice are represented as means \pm sd from four animals.

Table IV: Bone Marrow Myeloid: Erythoid Ratios

Group	Mouse #	Day 7	Day 14	Day 21
B-gal	1	1.43	2.12	5.78
	2	0.91	2.46	5.83
	3	1.62	1.03	3.66
	4		5.44	4.82
AVG		1.32	2.76	5.02
SD		0.37	0.37	1.89
B-gal	1	5.59	2.01	2.02
	2	6.51	1.25	2.13
	3	5.49	1.58	1.81
	4	0.50	2.51	2.92
AVG		4.52	1.86	2.22
SD-		1.29	2.72	0.56

All entries represent the number of myeloid cells per 1 erythroid cell. Normal mouse ratios are approximately 1:1 to 2:1.

Example 5

Additional Experiments Relating to

Hematopoietic Activity of Human CTLA-8

B18 (human CTLA-8) was tested for the ability to induce production of factors having hematopoietic activity in a factor-dependent cell proliferation assay using the

25

human erythroleukemic cell line, TF-1 (Kitamura et al., J. Cell Physiol. 140:323 (1989)). The cells were initially grown in the presence of rhGMCSF (100 U/ml). The cells were fed three days prior to setting up the assay. The assay conditions were as follows:

5	cells/well	5000/200µ1
	incubation time	3 days
	pulse time	4 hours
	amount of tritiated thymidine	0.5μCi/well
	counting time	1 minute
10	replicates	2

B18 alone, conditioned medium (CM) from B18 induced HS-5 cells were assayed. Buffer alone, CM from HS-5 cells induced with buffer and CM from uninduced HS-5 cells were assayed as controls. Results are shown in Fig. 5. B18 (human CTLA-8) demonstrated an abilit to induce production of factors which induced TF-1 proliferation. This activity was substantially eliminated by the addition of anti-GMCSF antibodies. These data demonstrate that human CTLA-8 (B18) is able to induce hematopoiesis. Particularly, without being bound by any theory, it appears that human CTLA-8 (B18) induces production of GM-CSF and/or IL-3.

20 <u>Example 6</u>

Ability of Human CTLA-8 to Induce Production of IL-6 and IL-8

MRC5 cells were incubated in the presence of human CTLA-8 (B18) and production of IL-6 and IL-8 were measured. Herpes CTLA-8 (IL-17) was used as a positive control. Applicants' human CTLA-8 (B18) demonstrated titratable production of both IL-6 and IL-8 (see Figs. 6 and 7).

All patent and literature references cited herein are incorporated by reference as if fully set forth.

SEQUENCE LISTING

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(i) APPLICANT: Jacobs, Kenneth Kelleher, Kerry Carlin, McKeough Goldman, Samuel Pittman, Debra Mi, Sha Neben, Steven Giannotti, JoAnn Golden'Fleet, Margaret

- (ii) TITLE OF INVENTION: Human CTLA-8 and Uses of CTLA-8-Related Proteins
 - (iii) NUMBER OF SEQUENCES: 9
 - (iv) CORRESPONDENCE ADDRESS:
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 (B) STREET: 87 CambridgePark Drive

 - (C) CITY: Cambridge
 - (D) STATE: Massachusetts
 - (E) COUNTRY: USA (F) ZIP: 02140

 - (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk

 - (B) COMPUTER: IBM PC compatible (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
 - (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
 - (viii) ATTORNEY/AGENT INFORMATION:

 - (A) NAME: Brown, Scott A.
 (B) REGISTRATION NUMBER: 32,724
 (C) REFERENCE/DOCKET NUMBER: GI5262
 - (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (617) 498-8224
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 - (2) INFORMATION FOR SEQ ID NO:1:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 813 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: cDNA
 - (iii) HYPOTHETICAL: NO
 - (ix) FEATURE:
 - (A) NAME/KEY: CDS
 (B) LOCATION: 56..544

	(xi)	SEC	QUENC	E DE	ESCRI	PTIC	ON: , S	EQ 1	D NO):1:						
GGG <i>I</i>	\AGA1	rac A	ATTC <i>i</i>	\C A G#	AA AC	GAGCT	rtcci	r GC#	CAAZ	AGTA	AGC	CACCA	AGC (GCAA(ATG Met 1	58
ACA Thr	GTG Val	AAG Lys	ACC Thr 5	CTG Leu	CAT His	GGC Gly	CCA Pro	GCC Ala 10	ATG Met	GTC Val	AAG Lys	TAC Tyr	TTG Leu 15	CTG Leu	CTG Leu	106
TCG Ser	ATA Ile	TTG Leu 20	GGG Gly	CTT Leu	GCC Ala	TTT Phe	CTG Leu 25	AGT Ser	GAG Glu	GCG Ala	GCA Ala	GCT Ala 30	CGG Arg	AAA Lys	ATC Ile	154
CCC Pro	AAA Lys 35	GTA Val	GGA Gly	CAT His	ACT Thr	TTT Phe 40	TTC Phe	CAA Gln	AAG Lys	CCT Pro	GAG Glu 45	AGT Ser	TGC Cys	CCG Pro	CCT Pro	202
GTG Val 50	CCA Pro	GGA Gly	GGT Gly	AGT Ser	ATG Met 55	AAG Lys	CTT Leu	GAC Asp	ATT Ile	GGC Gly 60	ATC Ile	ATC Ile	AAT Asn	GAA Glu	AAC Asn 65	250
CAG Gln	CGC Arg	GTT Val	TCC Ser	ATG Met 70	TCA Ser	CGT Arg	AAC Asn	ATC Ile	GAG Glu 75	AGC Ser	CGC Arg	TCC Ser	ACC Thr	TCC Ser 80	CCC Pro	298
TGG Trp	AAT Asn	TAC Tyr	ACT Thr 85	GTC Val	ACT Thr	TGG Trp	GAC Asp	CCC Pro 90	AAC Asn	CGG Arg	TAC Tyr	CCC Pro	TCG Ser 95	GAA Glu	GTT Val	346
GTA Val	CAG Gln	GCC Ala 100	CAG Gln	TGT Cys	AGG Arg	Asn	TTG Leu 105	GGC Gly	TGC Cys	ATC Ile	AAT Asn	GCT Ala 110	CAA Gln	GGA Gly	AAG Lys	394
GAA Glu	GAC Asp 115	ATC Ile	TCC Ser	ATG Met	AAT Asn	TCC Ser 120	GTT Val	CCC Pro	ATC Ile	CAG Gln	CAA Gln 125	GAG Glu	ACC Thr	CTG Leu	GTC Val	442
GTC Val 130	Arg	AGG Arg	AAG Lys	CAC His	CAA Gln 135	GGC Gly	TGC Cys	TCT Ser	GTT Val	TCT Ser 140	TTC Phe	CAG Gln	TTG Leu	GAG Glu	AAG Lys 145	490
GTG Val	CTG Leu	GTG Val	ACT Thr	GTT Val 150	Gly	TGC Cys	ACC Thr	TGC Cys	GTC Val 155	ACC Thr	CCT Pro	GTC Val	ATC Ile	CAC His 160	CAT. His	538
	CAG Gln		GAGG	TGC .	ATAT	CCAC	TC A	GCTG.	AAGA.	A GC	TGTA	GAAA	TGC	CACT	CCT	594
TAC	CCAG	TGC	TCTG	CAAC	AA G	TCCT	GTCT	G AC	ccc.	AATT	CCC	rcca	CTŢ (CACA	GGACTC	654
TTA	АТАА	GAC	CTGC	ACGG	AT G	GAAA	CAGA	A AA	TATT	CACA	ATG'	ratg'	rgt (GTAT	GTACTA	714
CAC	ATTT	ТАТ	TTGA	TATC	TA A	AATG	TTAG	G AG	AAAA.	ATTA	ATA'	TATT	CAG '	TGCT	AATATA	774
ΔΤΑ	ААСТ	АТТ	ААТА	АТТТ	AA A	AATA	AAAA	A AA	AAAA	AAA						813

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:

 (A) LENGTH: 163 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

	(x	i) S	EQUE	NCE	DESC	RIPT	'ION:	SEÇ	ID	NO: 2	:					
Met 1	Thr	Val	Lys	Thr 5	Leu	His	Gly	Pro	Ala 10	Met	Val	Lys	Tyr	Leu 15	Leu	
Leu	Ser	Ile	Leu 20	Gly	Leu	Ala	Phe	Leu 25	Ser	Glu	Ala	Ala	Ala 30	Arg	Lys	
Ile	Pro	Lys 35	Val	Gly	His	Thr	Phe 40	Phe	Gln	Lys	Pro	Glu 45	Ser	Cys	Pro	
Pro	Val 50	Pro	Gly	Gly	Ser	Met 55	Lys	Leu	Asp	Ile	Gly 60	Ile	Ile	Asn	Glu	
Asn 65	Gln	Arg	Val	Ser	Met 70	Ser	'Arg	Asn	Ile	Glu 75	Ser	Arg	Ser	Thr	Ser 80	
Pro	Trp	Asn	Tyr	Thr 85	Val	Thr	Trp	Asp	Pro 90	Asn	Arg	Tyr	Pro	Ser 95	Glu	
Val	Val	Gln	Ala 100	Gln	Cys	Arg	Asn	Leu 105	Gly	Cys	Ile	Asn	Ala 110	Gln	Gly	
Lys	Glu	Asp 115		Ser	Met	Asn	Ser 120	Val	Pro	Ile	Gln	Gln 125	Glu	Thr	Leu	•
Val	Val 130		Arg	Lys	His	Gln 135	Gly	Cys	Ser	Val	Ser 140	Phe	Gln	Leu	Glu	
Lys 145		Leu	Val	Thr	Val 150	Gly	Суѕ	Thr	.Cys	Val 155	Thr	Pro	Val	Ile	His 160	
His	Val	Glr	ì													
(2)	INF	ORM	ATION	FOF	SEÇ) ID	NO:3	:								
	(i	1	(A) I (B) 7 (C) S	ENGT TYPE: TRAN	CH: 4 : nuc NDEDI	ACTEF 161 h cleic NESS: : lir	oase aci dou	palı .d	:s							
	(ii	L) M	OLECT	JLE ?	TYPE	: cDi	AP								•	
	(ii	i) H	YPOTI	HETI	CAL:	ИО						•				
	(i:	x) F	EATU (A) (B)	NAME	/KEY TION	: CD:	s .455						٠			
						RIPT										
cc	ACC	ATG Met 1	TGC Cys	CTG Leu	ATG Met	CTG Leu 5	TTG Leu	CTG Leu	CTA Leu	CTG Leu	AAC Asn 10	CTG Leu	GAG Glu	GCT Ala	ACA Thr	47
Va	G AA 1 Ly 5		CA GC La Al	G GT a Va	ıl Le	C AT au Il	c cc e Pr	T CA	A AC n Se	ir Se	A GT er Va	G TG	T CC	A AA o As	C GCC n Ala 30	95
GA G1	G GC .u Al	C AI	AT AA	n Ph	TT CT ne Le 35	rc c <i>a</i> eu Gl	AG AA Ln As	ic G1	TT T	AG G1 /s Va 40	C AF	C CT	rg AA eu Ly	3 40	C ATC 1 Ile 15	143

AAC Asn	TCC Ser	CTT Leu	AGC Ser 50	TCA Ser	AAA Lys	GCG Ala	AGC Ser	TCG Ser 55	AGA Arg	AGG Arg	CCC Pro	TCA Ser	GAT Asp 60	TAC Tyr	CTC Leu	1	191
AAC Asn	CGT Arg	TCC Ser 65	ACT Thr	TCA Ser	CCC Pro	TGG Trp	ACT Thr 70	CTG Leu	AGC Ser	CGC Arg	AAT Asn	GAG Glu 75	GAC Asp	CCT Pro	GAT Asp	2	239
AGA Arg	TAT Tyr 80	CCT Pro	TCT Ser	GTG Val	ATC Ile	TGG Trp 85	GAG Glu	GCA Ala	CAG Gln	TGC Cys	CGC Arg 90	CAC His	CAG Gln	CGC Arg	TGT Cys	2	287
GTC Val 95	AAC Asn	GCT Ala	GAG Glu	GGG Gly	AAG Lys 100	TTG Leu	GAC Asp	CAC His	CAC His	ATG Met 105	AAT Asn	TCT Ser	GTT Val	CTC Leu	ATC Ile 110	,	335
CAG Gln	CAA Gln	GAG Glu	ATA Ile	CTA Leu 115	GTC Val	CTG Leu	AAG Lys	AGG Arg	GAG Glu 120	CCT Pro	GAG Glu	AAG Lys	TGC Cys	CCC Pro 125	TTC Phe	;	383
ACT Thr	TTC Phe	CGG Arg	GTG Val 130	GAG Glu	AAG Lys	ATG Met	CTG Leu	GTG Val 135	GGC Gly	GTG Val	GGC Gly	TGC C ys	ACC Thr 140	TGC Cys	GTT Val	•	431
			Val		CAT His				TAA					•			461

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 150 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
- Met Cys Leu Met Leu Leu Leu Leu Leu Asn Leu Glu Ala Thr Val Lys
- Ala Ala Val Leu Ile Pro Gln Ser Ser Val Cys Pro Asn Ala Glu Ala 20 25 30
- Asn Asn Phe Leu Gln Asn Val Lys Val Asn Leu Lys Val Ile Asn Ser 35 40 45
- Leu Ser Ser Lys Ala Ser Ser Arg Arg Pro Ser Asp Tyr Leu Asn Arg
 50 55 60
- Ser Thr Ser Pro Trp Thr Leu Ser Arg Asn Glu Asp Pro Asp Arg Tyr 65 70 75 80
- Pro Ser Val Ile Trp Glu Ala Gln Cys Arg His Gln Arg Cys Val Asn 85 90 95
- Ala Glu Gly Lys Leu Asp His His Met Asn Ser Val Leu Ile Gln Gln 100 105 110
- Glu Ile Leu Val Leu Lys Arg Glu Pro Glu Lys Cys Pro Phe Thr Phe 115 120 125
- Arg Val Glu Lys Met Leu Val Gly Val Gly Cys Thr Cys Val Ser Ser 130 140

Ile Val Arg His Ala Ser 145

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 459 base pairs

 - (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS (B) LOCATION: 1..453
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

	(XI)	SEÇ	CORNE		,,,,,,,,												
ATG Met 1	ACA Thr	TTT Phe	AGA Arg	ATG Met 5	ACT Thr	TCA Ser	CTT Leu	GTG Val	TTA Leu 10	CTT Leu	CTG Leu	CTG Leu	CTG Leu	AGC Ser 15	ATA Ile		48
GAT Asp	TGT Cys	ATA Ile	GTA Val 20	AAG Lys	TCA Ser	GAA Glu	ATA Ile	ACT Thr 25	AGT Ser	GCA Ala	CAA Gln	ACC Thr	CCA Pro 30	AGA Arg	TGC Cys		96
TTA Leu	GCT Ala	GCT Ala 35	AAC Asn	AAT Asn	AGC Ser	TTT Phe	CCA Pro 40	CGG Arg	TCT Ser	GTG Val	ATG Met	GTT Val 45	ACT Thr	TTG Leu	AGC Ser		144
ATC Ile	CGT Arg 50	AAC Asn	TGG Trp	AAT Asn	ACC Thr	AGT Ser 55	TCT Ser	AAA Lys	AGG Arg	GCT Ala	TCA Ser 60	Asp	TAC Tyr	TAC Tyr	AAT Asn		192
AGA Arg 65	TCT Ser	ACG Thr	TCT Ser	CCT Pro	TGG Trp 70	ACT Thr	CTC Leu	CAT His	CGC Arg	AAT Asn 75	GAA Glu	GAT Asp	CAA Gln	GAT Asp	AGA Arg 80		240
TAT Tyr	CCC Pro	TCT Ser	GTG Val	ATT Ile 85	TGG Trp	GAA Glu	GCA Ala	AAG Lys	TGT Cys 90	CGC Arg	TAC Tyr	TTA Leu	GGA Gly	TGT Cys 95	GTT Val		288
AAT Asn	GCT Ala	GAT Asp	GGG Gly 100	Asn	GTA Val	GAC Asp	TAC Tyr	CAC His	met	AAC Asn	TCA Ser	GTC Val	CCT Pro 110	116	CAA Gln		336
CAA Gln	GAG Glu	ATT Ile	Leu	GTG Val	GTG Val	CGC	AAA Lys 120	GLY	CAT His	CAA Gln	CCC Pro	TGC Cys 125	Pro	AAT Asn	TCA Ser	·	384
TTT Phe	AGG Arg 130	Lev	GAG Glu	AAG Lys	ATG Met	CTA Leu 135	. Val	ACT Thr	GTA Val	GGC Gly	TGC Cys	rnr	TGC Cys	GTT Val	ACT Thr	٠	432
CCC Pro	ATT Ile	GTT Val	CAC His	CAAT Asr	GTA 1 Val 150	. Asr	TAI	AA G									459

- (2) INFORMATION FOR SEQ ID NO:6:
 - (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 151 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Thr Phe Arg Met Thr Ser Leu Val Leu Leu Leu Leu Leu Ser Ile 1 10 15

Asp Cys Ile Val Lys Ser Glu Ile Thr Ser Ala Gln Thr Pro Arg Cys
20 25 30

Leu Ala Ala Asn Asn Ser Phe Pro Arg Ser Val Met Val Thr Leu Ser 35 40 . 45

Ile Arg Asn Trp Asn Thr Ser Ser Lys Arg Ala Ser Asp Tyr Tyr Asn 50 55 60

Arg Ser Thr Ser Pro Trp Thr Leu His Arg Asn Glu Asp Gln Asp Arg
65 70 75 80

Tyr Pro Ser Val Ile Trp Glu Ala Lys Cys Arg Tyr Leu Gly Cys Val 85 90 95

Asn Ala Asp Gly Asn Val Asp Tyr His Met Asn Ser Val Pro Ile Gln
100 105 110

Gln Glu Ile Leu Val Val Arg Lys Gly His Gln Pro Cys Pro Asn Ser 115 120 125

Phe Arg Leu Glu Lys Met Leu Val Thr Val Gly Cys Thr Cys Val Thr 130 135 140

Pro Ile Val His Asn Val Asp 145 150

- (2) INFORMATION FOR SEQ ID NO:7:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 28 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: cDNA
 - (iii) HYPOTHETICAL: NO
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CACAGGCATA CACAGGAAGA TACATTCA

- (2) INFORMATION FOR SEQ ID NO:8:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

 - (ii) MOLECULE TYPE: cDNA

			٠				 .		70	2.7	_	NIO
ľ	1	1	1	1	HY	PU	т.н	ET.	TC	ΑL	:	NO

(xi)	SEQUENCE	DESCRIPTION:	SEQ	ID	NO:8:	

TCTTGCTGGA TGGGAACGGA ATTCA

25

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 28 base pairs

 - (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATACATTCAC AGAAGAGCTT CCTGCACA

15

- 5 What is claimed is:
 - 1. An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:
 - (a) the nucleotide sequence of SEQ ID NO:1 from nucleotide 146 to nucleotide 544;
 - (b) a nucleotide sequence capable of hybridizing to a nucleic acid sequence specified in (a);
 - (c) a nucleotide sequence varying from the sequence of the nucleotide sequence specified in (a) as a result of degeneracy of the genetic code; and
 - (d) an allelic variant of the nucleotide sequence specified in (a).
 - 2. The polynucleotide of claim 1 wherein said nucleotide sequence encodes a protein having CTLA-8 activity.
- 3. The polynucleotide of claim 1 wherein said nucleotide sequence is operably linked to an expression control sequence.
 - 4. The polynucleotide of claim 1 comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 55 to nucleotide 544.
- 5. A host cell transformed with the polynucleotide of claim 3.
 - 6. The host cell of claim 5, wherein said cell is a mammalian cell.
 - 7. A process for producing a human CTLA-8 protein, said process comprising:
 - (a) growing a culture of the host cell of claim 5 in a suitable culture medium; and
 - (b) purifying the human CTLA-8 protein from the culture.

- 8. An isolated human CTLA-8 protein comprising an amino acid sequence selected from the group consisting of:
 - (a) the amino acid sequence of SEQ ID NO:2;
 - (b) the amino acid sequence of SEQ ID NO:2 from amino acids 11 to 163;
 - (c) the amino acid sequence of SEQ ID NO:2 from amino acids 29 to 163;
 - (d) the amino acid sequence of SEQ ID NO:2 from amino acids 31 to 163; and
 - (e) fragments of (a), (b), (c) or (d) having CTLA-8 activity.
 - 9. The protein of claim 8 comprising the amino acid sequence of SEQ ID NO:2.
- 10. The protein of claim 8 comprising the sequence from amino acid 29 to 163 of SEQ ID NO:2.
 - 11. A pharmaceutical composition comprising a human CTLA-8 protein of claim 8 and a pharmaceutically acceptable carrier.
 - 12. A human CTLA-8 protein produced according to the process of claim 7.
 - 13. A composition comprising an antibody which specifically reacts with a human CTLA-8 protein of claim 8.
- 14. A method of treating a mammalian subject comprising administering a therapeutically effective amount of a composition of claim 11.
- 15. A method of treating a mammalian subject comprising administering a therapeutically effective amount of a composition comprising a pharmaceutically acceptable carrier and a protein comprising an amino acid sequence selected from the group consisting of:
 - (a) the amino acid sequence of SEQ ID NO:4;

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- (b) the amino acid sequence of SEQ ID NO:4 from amino acids 18 to 150; and
- (c) fragments of (a) or (b) having CTLA-8 activity.
- 16. The method of claim 15 wherein said protein comprises the amino acid sequence of SEQ ID NO:4.

17. The method of claim 15 wherein said protein comprises the amino acid sequenceof SEQ ID NO:4 from amino acids 18 to 150.

- 18. A method of treating a mammalian subject administering a therapeutically effective
 amount of a composition comprising a pharmaceutically acceptable carrier and a protein
 comprising an amino acid sequence selected from the group consisting of:
 - (a) the amino acid sequence of SEQ ID NO:6;
 - (b) the amino acid sequence of SEQ ID NO:6 from amino acids 19 to 151; and
 - (c) fragments of (a) or (b) having CTLA-8 activity.

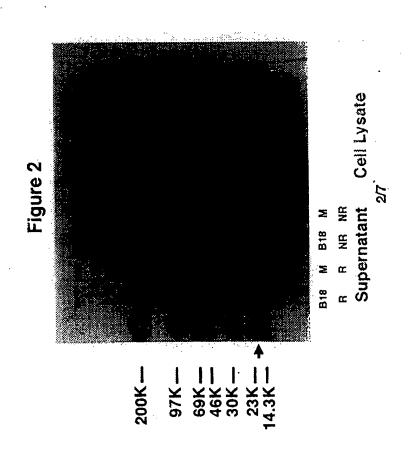
19. The method of claim 18 wherein said protein comprises the amino acid sequence of SEQ ID NO:6.

- 20. The method of claim 18 wherein said protein comprises the amino acid sequence of SEQ ID NO:6 from amino acids 19 to 151.
 - 21. The polynucleotide of claim 1 comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 86 to nucleotide 544.
- The protein of claim 8 comprising the sequence from amino acid 11 to 163 of SEQ ID NO:2.

- 5 23. A method of treating a mammalian subject comprising administering a therapeutically effective amount of a composition comprising a pharmaceutically acceptable carrier and IL-17 or an active fragment thereo.
- 24. The method of claim 14, 15, 18 or 23 wherein said subject is treated to produce an effect selected from the group consisting of inhibition of angiogenesis, inhibition of growth or proliferation of vascular endothelial cells, inhibition of tumor growth, inhibition of angiogenesis-dependent tissue growth, proliferation of myeloid cells or progenitors, proliferation of erythroid cells or progenitors, proliferation of lymphoid cells or progenitors, induction of IFNγ production, induction of IL-3 production and induction of GM-CSF production.
 - 25. The composition of claim 3 wherein said polynucleotide is contained in a vector suitable for *in vivo* expression in a mammalian subject.
- 26. The polynucleotide of claim 1 comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 139 to nucleotide 544.
 - 27. The protein of claim 8 comprising the sequence from amino acid 31 to 163 of SEQ ID NO:2.

5 1 5 3	ਜ ਜ ਲ ਜ ਜ ਜ	133 133 135
-2IVRSEITISAO-TPRCL-AANNSPPRSVMVTUSIRNWNTSSKRASDYYNRSTSP a 8AVLIPO-SSVCPNABANNFLONVKVNLKVINSLSSKASSRRPSDYLNRSTSP F1 AARKIPKVGHTFFQKPESCPPVPGGSMKLDIGIINENQRVSMSRNIESRSTSP	- 2 WTLHRNEDQDRYPSVIWEAKCRYLGCVNADGNCDYHMNSVPIQQEILVVRKGHQPCPNSF1111 WTLSRNEDPDRYPSVIWEAQCRHQRCVNAEGKLDHHMNSVLIQQEILVLKREPEKCPFTF111 F1 WNYTVTWDPNRYPSEVVQAQCRNLGCINAQGKEDISMNSVPIQQETLLVVRRKHQGCSVSF113	RLEKMLVTVGCTCVTPIVHNUD RUEKMLVGVGCTCVSSIVRHAS QLEKVLVTVGCTCVTPVIHHVQ
Havie 2 lusctla8 B18_F1	и в в в в в в в в в в в в в в в в в в в	Hsvie_2 usctla8 .B18_F1
# Z	# 2	= 2

Fig. 1



2/7

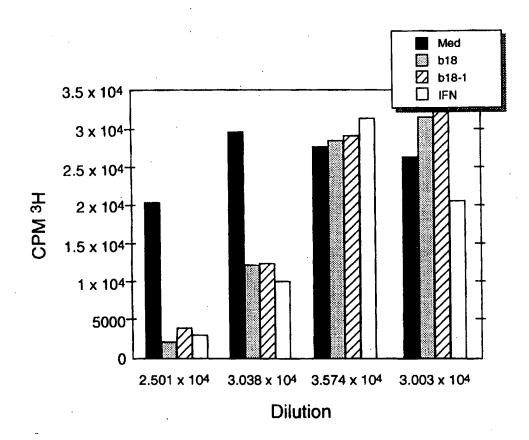
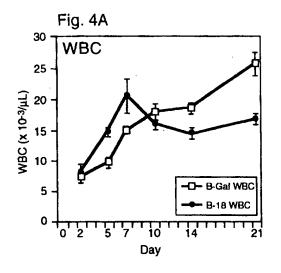
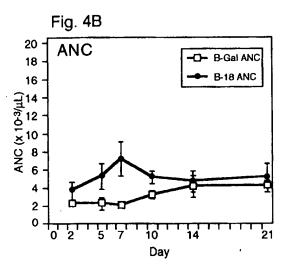
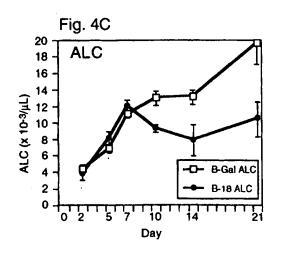
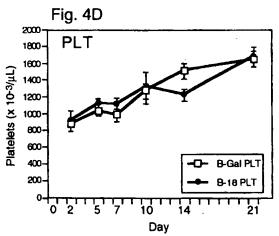


Fig. 3









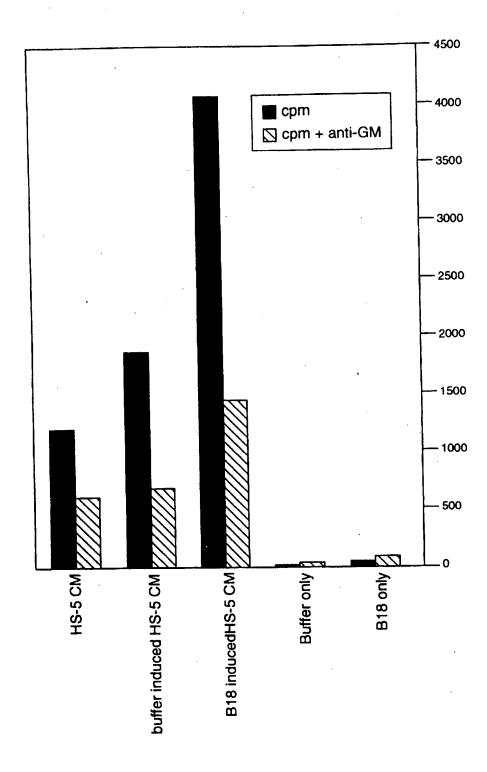
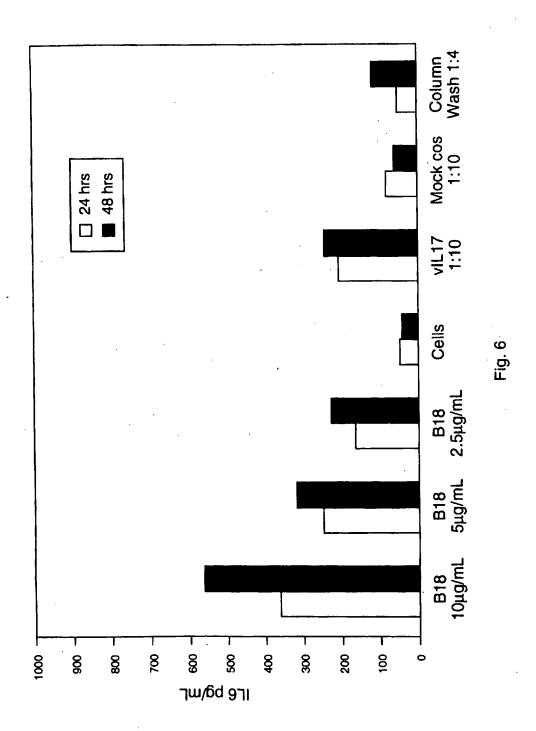
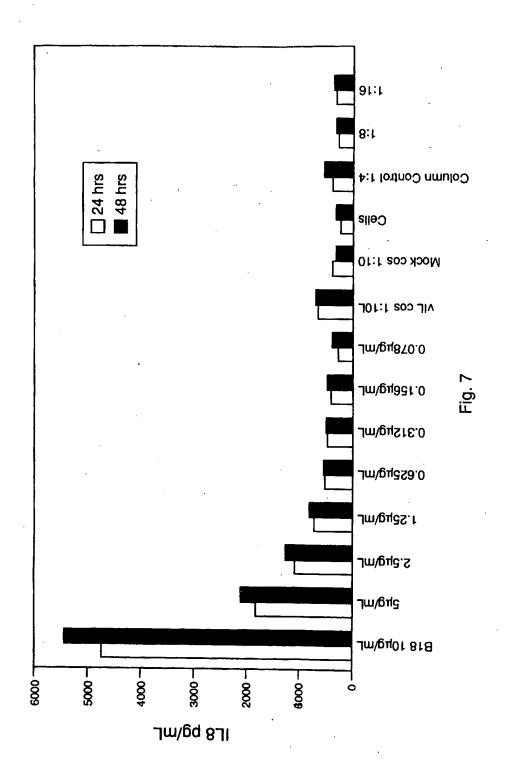


Fig. 5 5/7

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SUBSTITUTE SHEET (RULE 26)



r ational Application No PCT/US 96/11889

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According to	FICATION OF SUBJECT MATTER C12N15/19 C12N15/12 C12N15 C07K14/54 A61K38/17 A61K38 C07K16/24 C07K16/28 A61K48 of International Patent Classification (IPC) or to both national classification system followed by classification commentation searched (classification system followed by classification C12N C07K A61K	ssalication and IPC	/52 C07K /20 C12N	14/725 5/10
	tion searched other than minimum documentation to the extent th			
C. DOCUM	MENTS CONSIDERED TO BE RELEVANT Citation of document, with indication, where appropriate, of the	e relevant passages		Relevant to claim No.
X	WO 95 18826 A (SCHERING CORP ; I SANTE RECH MED (FR)) 13 July 19 cited in the application	NST NAT 95		1-3,5-7, 12, 15-20, 23-25
A	see page 3, line 21 - page 4, l see page 12, line 1 - page 16, see page 30, line 1-13 Seq.ID:2 see page 53 Seq.ID:4 see page 55 Seq.ID:5 see page 56 Seq.ID:7/8 see page 58 - page 59	ine 11 line 6		11,13,14
X Fu	ther documents are listed in the continuation of box C.	X Patent famil	y members are listed	in annex.
* Special categories of cited documents: 'A' document defining the general state of the art which is not considered to be of particular relevance 'E' earlier document but published on or after the international filing date 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) 'O' document referring to an oral disclosure, use, exhibition or other means 'P' document published prior to the international filing date but later than the priority date claimed Date of the actual completion of the international search		or prionty date cited to underst invention 'X' document of par cannot be consi involve an inversion of par cannot be consi document of par cannot be consi document is con ments, such cor in the art. '&' document memi	 'X' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is taken alone 'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled 	
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentiaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo ni, Fax: (+ 31-70) 340-3016			Authorized officer Macchia, G	



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PCT/US 96/11889

	TO BE DELEVANT	
	tion) DOCUMENTS CONSIDERED TO BE RELEVANT Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
ategory *	Citation of document, with indicated,	15.20
	THE JOURNAL OF IMMUNOLOGY, vol. 150, no. 12, 15 June 1993, pages 5445-5456, XP002035505 ROUVIER E. ET AL.: "CTLA-8, cloned from an activated T cell, bearing AU-rich messenger RNA instability sequences, and homologous to a Herpesvirus Saimiri gene" cited in the application see page 5445 - page 5446, line 11 see page 5449, right-hand column, line 26 - page 5450 see page 5451; figure 3 see page 5453, left-hand column, line 10 - right-hand column, line 16	15-20
X	JOURNAL OF VIROLOGY,	1
A ,	pages 5047-5058, XP000615399 ALBRECHT J -C ET AL: "PRIMARY STRUCTURE OF THE HERPESVIRUS SAIMIRI GENOME" Seq.ID:1 from nt.552 to nt.217 (reverse orientation) is 61.4% homologous to X64346 from nt.26931 to nt.27266.	18-20
	see page 5048, right-hand column, line 15-18 ORF13 see page 5049; table 1	1-14,21,
E	WO 97 07198 A (GENETICS INST) 27 February 1997 see page 8, line 15-31 see page 9, line 26 - page 11, line 7 see page 13, line 16-27 see page 18, line 10 - page 19, line 25 see page 25, line 11-15 see page 27, line 5-17 see page 31, line 27-34 Seq.ID:11-12 see page 49 - page 51	22,24-27
P,X	JOURNAL OF IMMUNOLOGY, vol. 155, no. 12, 15 December 1995, pages 5483-5486, XP000602481 YAO Z ET AL: "HUMAN IL-17: A NOVEL CYTOKINE DERIVED FROM T CELLS" see page 5483 see page 5484; figure 1	1-3,5-7,
	-/	



onal Application No PCT/US 96/11889

C.(Continue	tion) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	
P,X	THE JOURNAL OF EXPERIMENTAL MEDICINE, vol. 183, no. 6, 1 June 1996, pages 2593-2603, XP002035506 FOSSIEZ F. ET AL.: "T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines" see abstract see page 2594; figure 1	1-3,5-7, 15-20, 23,24	
P,X	IMMUNITY, vol. 3, no. 6, 1 December 1995, pages 811-821, XP000579309 YAO Z ET AL: "HERPESVIRUS SAIMIRI ENCODES A NEW CYTOKINE, IL-17, WHICH BINDS TO A NOVEL CYTOKINE RECEPTOR" see page 811 see page 815, left-hand column, line 50 - page 818, left-hand column	15-20, 23,24	
Ε	WO 96 29408 A (IMMUNEX CORP) 26 September 1996 see page 1, line 1 - page 2, line 33 Seq.ID:8 see page 36	18-20	
P,X	GENE, vol. 168, no. 2, 12 February 1996, pages 223-225, XP002035631 YAO ET AL.: "Complete nucleotide sequence of the mouse CTLA8 gene" see page 225; figure 2	1,2	



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/11889

Box I	Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)
This Inte	ernational Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
t. X	Claims Nos.: 14-20,23,24 because they relate to subject matter not required to be searched by this Authority, namely: Remark: Although claims 14-20,2,24 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2.	Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3.	Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II	Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This in	ternational Searching Authority found multiple inventions in this international application, as follows:
Se	ee continuation-sheet
۱. 🗀	As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. X	As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3	As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. [No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Rema	The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

- 1) claims 1-14, 21, 22, 25-27 all totally; claim 24 partially.

 Isolated polynucleotide comprising Seq.ID:1, homologue sequences and derivates. Vectors and transformed host cells. Process for producing human recombinant CTLA-8 protein. Isolated human CTLA-8 protein as in Seq.ID:2 and fragments. Pharmaceutical compositions and uses in therapy. Antibodies.
 - claims 15-17 all totally; claim 24 partially.
 Therapeutical uses of the protein as in Seq.ID:4 or fragments.
 - 3) claims 18-20 all totally; claim 24 partially.

 Therapeutical uses of the protein as in Seq.ID:6 or fragments.
 - 4) claims 23 all totally; claim 24 partially.

 Therapeutical uses of IL-17 or active fragments.

INTERNATIONAL SEARCH REPORT

national Application No PCT/US 96/11889

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9518826 A		AU 1520895 A EP 0733069 A JP 9501572 T	01-08-95 25-09-96 18-02-97
WO 9707198 A	27-02-97	AU 6712396 A AU 6768596 A WO 9704097 A	18-02-97 12-03-97 06-02-97
WO 9629408 A	26-09-96	AU 5526396 A	08-10-96